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THE ROLE OF 5-HT_2 RECEPTOR SUBTYPES IN THE CONTROL OF MICTURITION IN URETHANE ANAESTHETIZED FEMALE RATS

By

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Abstract

Central 5-HT containing pathways have been shown to play an important role in the control of micturition with a focus on 5-HT receptor influence on the parasympathetic outflow to the bladder, and somatic outflow to the external urethral sphincter (EUS) in the rat. 5-HT_{1A} and 5-HT_7 receptors have been revealed to be physiologically involved in the control of micturition. Moreover, from the literature, activation of the 5-HT_2 receptor, specifically 5-HT_{2C} receptor has been observed to be inhibitory on the micturition reflex, although no physiological role for this receptor subtype has yet been established. Using reportedly selective 5-HT_2 receptor agonists and antagonists, the present work reveals the excitatory actions of the 5-HT_{2A} receptor on the external urethral sphincter and the micturition reflex, the inhibitory actions of the 5-HT_{2C} receptor in micturition and the involvement of 5-HT_{2B} receptors in mediating urethral smooth muscle contraction. Further, data from the present study demonstrates that 5-HT_{2A} receptors excite the external urethral sphincter at the level of the sacral spinal cord and further supports the view that the inhibitory action of the 5-HT_{2C} receptors on micturition is centrally mediated. Molecular evidence regarding expression of 5-HT_2 receptor subtypes in the lower urinary tract of the rat is also provided in the present study, with 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptor mRNA expression observed in the rat bladder, urethra and brain respectively.

Overall, this thesis provides evidence for the involvement of 5-HT_2 receptors in the control of micturition, although it is still unclear as to the physiological role of this receptor family in the control of micturition.

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Introduction

1 Introduction

Urine is produced in the kidneys continuously and for a practical reason is first collected in the bladder where it is stored until disposal is possible. The lower urinary tract mainly functions in continence to store and void urine when convenient, and plays a role in sexual function in males. In many animals, micturition does not take place at random, but is part of a rather complicated behaviour, directly related to the survival of the individual or species, where urine is used for territorial marking or sexual function (Blok & Holstege, 1999).

Urine storage and its eventual expulsion from the body involves a complex pattern of efferent and afferent signalling in both the autonomic and somatic nervous systems which are dependant on central nervous system control. A great interest in the lower urinary tract is attributed to the fact that several pathological conditions exist particularly in the western world where development of pharmacological therapies for the treatment of these conditions is of great focus both in the pharmaceutical industry and academia. Part of the focus on these pharmacological therapies is from gaining a better understanding of the mechanisms by which neurotransmitters such as monoamines, amino acids and peptides are involved in the control of the lower urinary tract. This thesis will therefore focus on the monoamine neurotransmitter 5-Hydroxytryptamine (5-HT), primarily concentrating on the 5-HT_2 receptor subtype and its role in both bladder and urethral function.

1.1 The lower urinary tract

The lower urinary tract (LUT) which comprises of the bladder and urethra serves to store and periodically eliminate urine. For this process to occur, close coordination between these two components is required in order for continence to be maintained and timely micturition to occur. The bladder forms a reservoir for urine storage, receiving urine from the ureters, but never under normal conditions, allowing it to flow back into the upper urinary tract (Lincoln & Burnstock, 1993). The urethra connects the bladder to the exterior and acts as an outlet, thus eliminating urine from the body (see de Groat & Yoshimura, 2001).

1.1.1 Bladder structure and function

The bladder consists of two main components – the bladder body, located above the ureteral orifices and the bladder base, consisting of the trigone, urethrovesical junction, detrusor and bladder wall. The bladder can be divided into two distinct regions – the *detrusor* and the *trigone.* The *detrusor* forms the smooth muscle of the bladder, is highly distensible and receives a parasympathetic input. Contraction of the detrusor muscle encourages expulsion of urine from the bladder. The *trigone* on the other hand is located dorsally at the bladder neck and undergoes little change in size during filling. The *trigone* is thought to prevent backflow of urine into the ureters following an increase in bladder pressure during voiding. It has been proposed that a sympathetic supply to the *trigone* aids in preventing this backflow into the ureters.

1.1.2 Urothelium

The urothelium is a layer of epithelial cells lining the surface of the urinary bladder and is composed of at least three layers which include; a basal cell layer attached to a basement membrane, an intermediate layer, and a superficial (or apical) layer composed of large hexagonal cells which are also known as "umbrella cells". In addition to functioning as a barrier of fluid and ion transport (see Lewis, 2000), experimental and clinical evidence has shown the urothelium to be a highly specialized structure involved in the micturition reflex, as it increases the size of the umbrella cells during the filling phase, as well as releasing mediators that activate sensory fibers (Birder *et al.*, 2001). Additionally, the urothelium has been implicated in metabolic secretion, regulation of inflammation and afferent functions. Molecules expressed by the urothelium include receptors for bradykinin (Chopra *et al.*, 2005), neurotrophins (Wolf-Johnston *et al.*, 2004), purines (Lee *et al.*, 2000; Tempest *et al.*, 2004), noradrenaline (Birder *et al.*, 1998, 2002b) as well as chemical mediators and ion channels such as adenosine triphosphate (ATP), acetylcholine (ACh), nitric oxide (NO), transient receptor potential vallinoid 1 (TRPV1) and potassium (K) channels respectively. These multiple actions of the urothelium have yielded a lot of attention in present urological research especially as modifications of the urothelium in a number of pathological conditions can result in passage of unwanted urinary constituents, or the release of neuroactive substances that may lead to sensory nerves and sensory symptoms such as urinary frequency and urgency (see Birder & de Groat, 2007). Additionally, a correlation has been observed between interstitial cystitis (IC) and urothelial TRPV1 expression thus making the urothelium a possible target in the treatment of this condition (Burcher, 2005). Therefore, in present day urology research

the urothelium is a great target for pharmacological interventions aimed at either the urothelial receptor and ion channel expression or neurotransmitter release mechanisms. One current mode of treatment targeting the urothelium is through injection of botulinium toxin A (BTX-A) into the bladder wall to prevent detrusor overactivity (Smith & Chancellor, 2004; Schurch *et al.*, 2005). It has been hypothesised that the BTX-A acts by blocking the release of ATP (Barrick *et al.*, 2004) which would normally trigger sensations of fullness and pain, or induce reflex changes in bladder activity (Burnstock, 2001). Additionally, BTX-A acts to reduce activation of afferent nerves by bladder irritation (Chuang *et al.*, 2004; Vemulakonda *et al.*, 2005). The urothelium therefore provides important avenues that could lead to new strategies for the clinical management of bladder disorders.

1.1.3 Urethral structure and function

The mammalian urethra is a highly complex tubular organ that connects the bladder to the exterior. In the human male, the urethra is divided into the bladder neck, prostatic, membranous, bulbar and penile sections with only the first three contributing to urinary continence (see Brading, 1999). In the human female, the urethra is more uniform in structure and is much shorter in length. A cross section of the human female urethra shows a thick, internal band of longitudinal smooth muscle lying just under the submucosa, a thin, middle band of circular smooth muscle and an omega-shaped, external band of striated muscle also referred to as the rhabdosphincter (see Thor, 2004). A similar arrangement has been described in the female rat urethra (Hutch & Rambo, 1967). It has been suggested that longitudinal and circular smooth muscles play different but

important roles in voiding where the former shortens the proximal urethra as well as opening the bladder neck and the latter functions to close the urethra as well as increasing urethral resistance to urine flow (see Andersson, 1993; Hassouna *et al.*, 1983; Mattiasson *et al.*, 1989). Smooth muscles traverse the entire length of the urethra in the female and up to and including the membranous urethra in the male. The external urethral sphincter contains an intramural layer of striated muscle that is separate from the periurethral skeletal muscle of the pelvic floor (see Brading, 1999). In the human male, the striated muscle extends from the base of the bladder and the anterior aspect of the prostrate along the full length of the membranous urethra whereas in the human female, the striated muscle extends from the proximal urethra distally. The smooth and striated muscle fibers are intermingled in the middle circular smooth muscle layer (Watanabe & Yamamoto, 1979) and it has been suggested that these two types of muscles are closely linked functionally as they have been shown to contribute to urethral closure pressure.

During bladder filling, the main function of the urethra is to prevent leakage of urine from the bladder by having a urethral closure pressure that exceeds the intravesical pressure. If the pressure along the length of the urethra is measured in both animals and man at rest during the filling stage of the micturition cycle, a low urethral pressure is found to exist at the bladder neck, and this rises to a peak pressure in the mid urethra which declines towards the urethral opening (see Brading, 1999). During micturition, the pressure in the urethra drops thus relaxing the muscles generating intraurethral pressure. However, this is not enough to open the urethral lumen and therefore together with a drop in urethral pressure, the urethra shortens and funnelling of the bladder neck occurs. This therefore actively prepares the urethra to allow urine flow.

1.1.4 Comparisons between mammalian species used for urological research

Several mammalian species have been used as models in urological research when investigating potential therapeutic targets for dysfunctions of the lower urinary tract. Species such as pigs, dogs, rats and guinea pigs have been greatly used both in the academic and industrial setting. The female pig has been identified as an excellent model of bladder outflow obstruction as the pig produces a progressive obstruction that is similar to detrusor instability in humans (Sibley, 1985). However, anatomically, the female pig urethra is quite different to that of the female human urethra, as the striated muscle in the pig is only present in the distal region (Dass *et al.*, 2001) of the urethra, whereas 80% of the urethral length in humans is covered by striated muscles (DeLancy, 1994). On the other hand, the length of the smooth and striated muscle in the guinea pig urethra bears a closer structural resemblance to humans. Functionally, external urethral sphincteric activity in guinea pigs and humans is very similar. In both species, external urethral sphincteric activity is low during the early filling phase of the bladder, with gradual rises in activity observed as the bladder volume increases. This activity is however completely inhibited immediately prior to and during bladder emptying, reappearing when voiding is completed (Walters *et al.*, 2006). This phenomenon of sphincteric activity 'switch off' (also known as the guarding reflex) prior to and during bladder emptying is not observed in the rat, thus demonstrating the existence of species differences in the actions of the striated urethral muscle. It therefore appears that for efficient voiding to occur in the different species, either a 'switch off' in sphincteric activity immediately prior to contraction of the bladder (humans and guinea pigs), or

appearance of sphincteric activity on the initiation of micturition (rodents) is essential. With regards to the voiding characteristics similarities and differences have been observed in the different species. For example, both the rat and human display similar patterns of flow and peak bladder pressure (Schmidt *et al.*, 2003), whereas only the rat displays oscillatory activity on bladder pressure during voiding (Van Asselt *et al.*, 1995). It is therefore important to note that the choice of which species to use as a comparative animal model for humans is primarily dependant on the research interest.

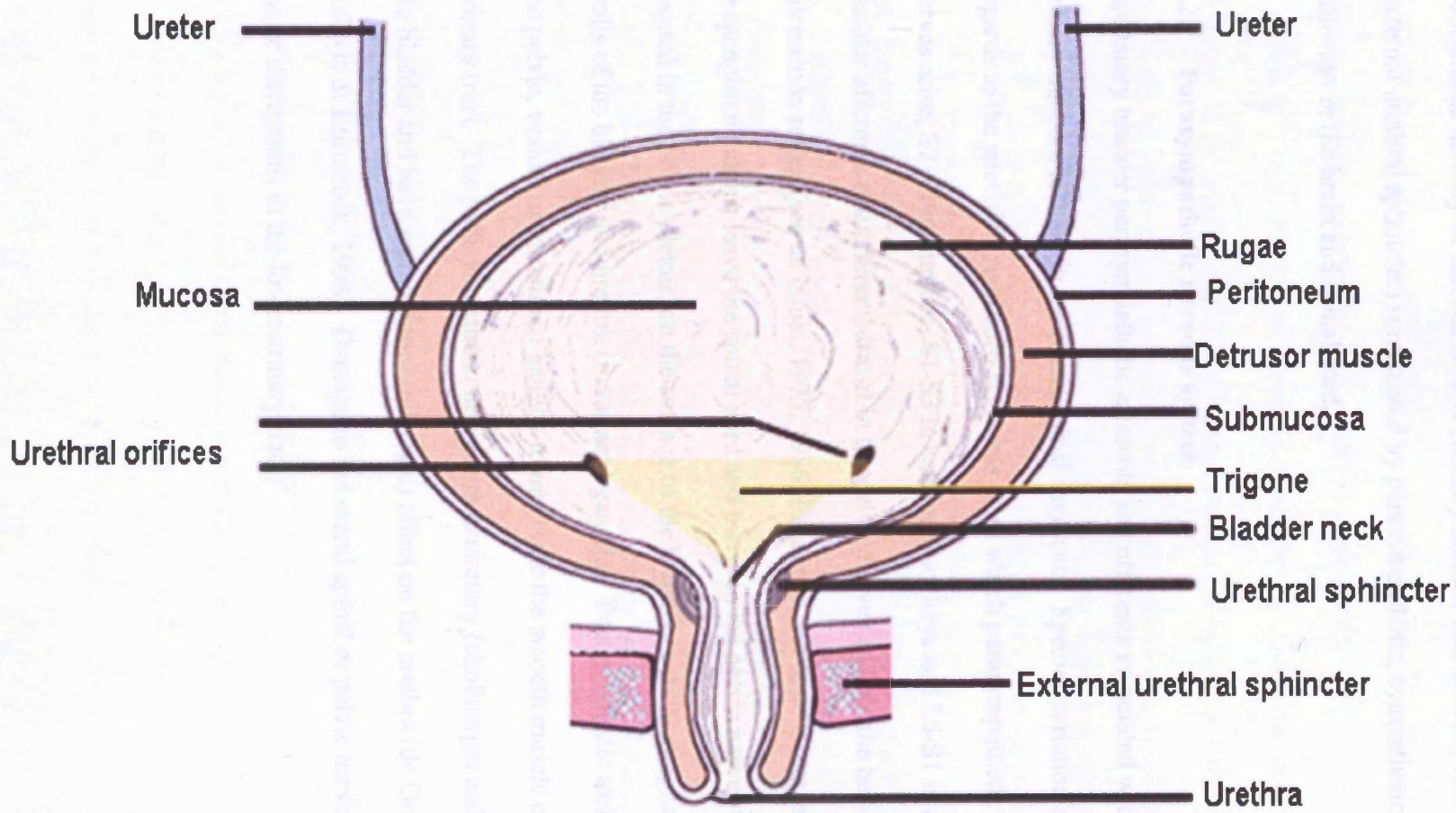

Figure 1.1 Diagrammatic representation of the female bladder and urethra. (Modified from www.Ivy-Rose.co.uk)

1.2 Autonomic innervation of the lower urinary tract

Coordinated activity of smooth muscle (urinary bladder and urethra) and striated muscle (external urethral sphincter) is mediated by parasympathetic, sympathetic and somatic pathways in the brain and spinal cord.

1.2.1 Parasympathetic nervous system

Excitatory bladder parasympathetic afferents and efferents associated with the lower urinary tract are located in the sacral spinal segments. Species variation exists with regards to the specific spinal cord segments from which parasympathetic preganglionic nerves arise; S2-S4 in humans, S1-S3 in cats and monkeys and L6-S1 in rats. These bladder afferents and efferents travel in the pelvic nerves and are the basis for the micturition reflex (see de Groat, 1975, 1986; Elbadawi, 1996; de Groat *et al.*, 1999). Preganglionic axons leave the spinal cord and travel in the pelvic nerves to ganglia located in the pelvic plexus, on the surface of the bladder (vesical ganglia) or within the walls of the bladder and urethra (intramural ganglia). Postganglionic axons then leave the pelvic, vesical or intramural ganglia to innervate the smooth muscle of the lower urinary tract. The parasympathetic nerves are excitatory (cholinergic and purinergic) to the bladder and have an inhibitory (nitrergic) effect on the urethra (de Groat *et al.*, 1993; Ralevic & Burnstock, 1998). Damage to the sacral spinal or pelvic nerves results in major disruptions to the lower urinary tract.

1.2.2 Sympathetic nervous system

The sympathetic outflow to the bladder and urethra originates in the lower thoracic and upper lumbar spinal segments (T10-L2) and travels via the sympathetic chain to the inferior mesenteric ganglia and then via the hypogastric nerves to the pelvic plexus of the lower urinary tract. These sympathetic pathways which release noradrenaline are excitatory to the bladder neck and the proximal urethra and they provide both facilitatory and inhibitory inputs to the parasympathetic ganglia (Andersson, 1993; de Groat *et al.*, 1993). The sympathetic system is active between voids with the primary role of facilitating bladder filling via β adrenergic receptor-mediated relaxation of the bladder smooth muscle and α-adrenergic receptor mediated contraction of the smooth muscle of the bladder neck and the urethra (see Andersson, 2000). It is also thought that the sympathetic nervous system plays a role in inhibiting parasympathetic activity during the accommodation phase of urine storage. Disruption to the sympathetic outflow to the bladder (although not life threatening) may result in a decrease in the competency of the bladder neck and proximal urethra as well as a decrease in bladder storage volumes, thus affecting continence.

1.2.3 Somatic nervous system

Motorneurones located in the Onuf's nucleus are responsible for innervation of the urethral striated muscle and, to a large extent the control of urethral function via postsynaptic nerves. These motorneurones primarily receive a somatic input. Studies have revealed the existence of a species variation with regards to the location of the Onuf's nucleus. In mammalian species such as hamster (Gerrits *et al.*, 1997), dog

(Kuzuhara *et al.*, 1980; Petras & Cummings, 1978), cat (Kuzuhara *et al.*, 1980) and monkey (Nakagawa, 1980; Roppolo *et al.*, 1985), the Onuf's nucleus is located in the ventrolateral aspect of the ventral horn. In the rat, however, the Onuf's nucleus consists of two separate cell groups, a dorsomedial group and a ventrolateral group. Structurally, motorneurones innervating the external urethral sphincter are different from other motorneurones as they are smaller in size and they possess bundled dendrites responsible for strong synchronous activation or inhibition (see Nitti, 2004). Innervation of the striated muscle is largely via the motor branch of the pudendal nerve (McKenna & Nadelhaft, 1986; Thor *et al.*, 1989; Dubrovsky & Filipini, 1990; Vanderhorst & Holstege, 1997) which provides an excitatory cholinergic input to the striated muscle of the urethral sphincter. However, a GABAergic/glycinergic inhibitory interneuronal mechanism also exists (see Fraser & Chancellor, 2003). The somatic nervous system is tonically active during bladder filling following activation of pudendal motorneurones, and has been found to play a major role in continence by maintaining closure of the urethral sphincter. Challenges to continence such as sneezing, coughing or postural changes are thought to be mediated by the somatic nervous system. Damage caused to the pudendal nerve could thus result in a hypoactive urethral sphincter and this may eventually lead to conditions such as stress urinary incontinence.

1.2.4 Pelvic ganglia

Pelvic ganglia are clusters of autonomic ganglion neurones that are the final motor pathways mediating storage and voiding functions of the lower urinary tract, bowel and reproductive behaviours (see Keast, 2006). The pelvic ganglia provide a mixture of sympathetic and parasympathetic neurones where some neurones receive synaptic inputs from the lumbar spinal cord and some neurones receive inputs from the sacral spinal cord. In some species, there are pelvic ganglia that receive inputs from both lumbar and sacral spinal cord. Sympathetic neurones in the pelvic plexus tend to occur more centrally whereas parasympathetic neurones are located more dorsally. An interesting feature of pelvic neurones is that pelvic parasympathetic neurones unlike other parasympathetic neurones lie quite some distance from their target tissues. Although a disadvantage during common surgical procedures such as prostatectomy and hysterectomy, this unique neuroanatomical feature makes these neurones easily accessible for experimental studies. Interspecies differences exist with regards to the structure of the pelvic ganglia. Rodents tend to have large, paired pelvic ganglia whereas in humans, cats, rabbits, pigs and dogs, the pelvic ganglia consist of a complicated meshwork of fibres. Overall, the pelvic ganglia play an important role in the coordination and integration of lower urinary tract function.

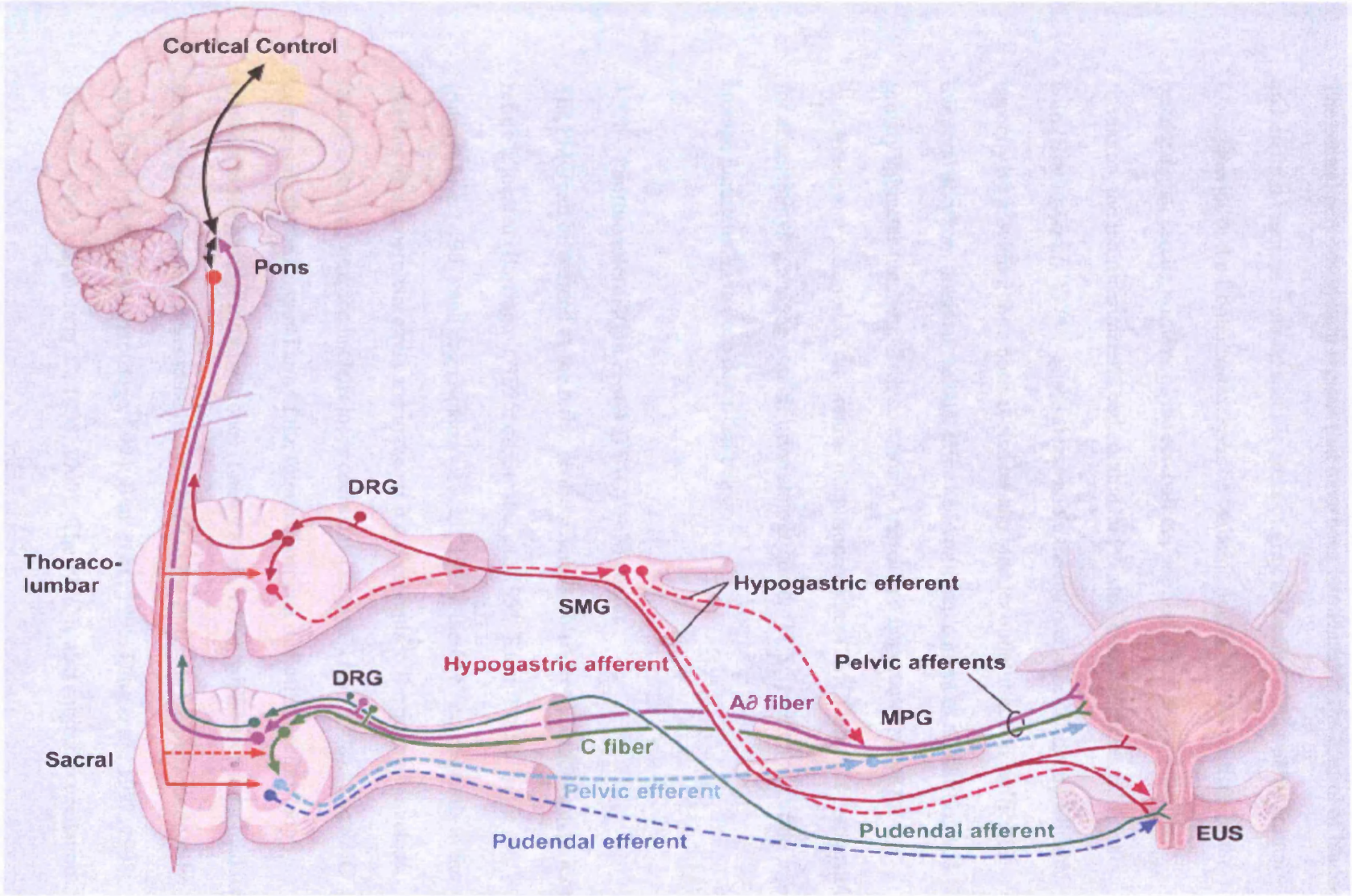

Figure 1.2 Schematic representation of the innervation of the lower urinary tract (Ford *et al.*, 2006).
DRG, dorsal root ganglia; MPG, major pelvic ganglion; SMG, superior mesenteric ganglion; EUS, external urethral sphincter

1.3 Central control of micturition and urine storage

The central nervous system regions that have been implicated in the control of bladder and urethral function include; specific areas of gray and white matter of the frontal lobes anterior to the frontal cortex, specific thalamic nuclei, several portions of the basal ganglia, certain portions of the cerebellum, pontine-mesenphalic reticular formation, the intermediolateral cell column and ventral horns of the sacral spinal cord (Bradley *et al.*, 1974). These areas enable the maintenance of adequate bladder capacity by providing for a normal bladder and bladder outlet function, sufficient temporal duration, minimal residual urine and volitional control of micturition and urinary sphincter function. Two commonly known and important centers for micturition and continence, the pontine micturition center (PMC) which is responsible for urine emptying and the pontine urine storage center (PUSC) which is a urine storage facilitator have been described below.

1.3.1 Pontine micturition center (PMC) or M region

The PMC can be defined as the reflex centre where the bladder-to bladder micturition reflex is located (Barrington, 1925; de Groat *et al.*, 1993; Kuru, 1965). In the cat (Sugaya *et al.*, 1987) and dog (Nishizawa *et al.*, 1988), the PMC corresponds to the nucleus locus coeruleus alpha and in the rat it corresponds to Barrington's nucleus, which is located near the nucleus locus coeruleus (Sugaya *et al.*, 1998). The PMC comprising the bulbospinal arm of the spinobulbospinal micturition reflex loop projects to the bladder motorneurones (Loewy *et al.*, 1979), whilst ascending bladder sensory information appears to be relayed back to the PMC via the periaqueductal gray (Liu, 1983; Blok & Holstege, 1994; Blok *et al.*, 1995; Ding *et al.*, 1997, 1998; Marson, 1997; Matsuura *et al.*, 1998, 2000). The PMC is also subject to excitation

and inhibition from other brainstem, subcortical and cortical regions such as cerebellum, periaqueductal gray, substantia nigra, red nucleus, thalamus, hypothalamus, amygdaloid body and the cerebral cortex (Sugaya *et al.*, 1988c). Electrical or chemical stimulation of the PMC produces a sharp decrease in urethral pressure, pelvic floor relaxation and an increase in intravesical pressure (Holstege *et al.*, 1986; Mallory *et al.*, 1991). The PMC is therefore thought to act as a switch between continence and micturition, where it seems to regulate bladder capacity and bladder contraction as well as coordinating the activity between the bladder and the urethral sphincter.

1.3.2 Pontine urine storage center (PUSC) or L region

Anterograde axonal tracing studies in the cat established that the supraspinal circuitry controlling the urethral striated muscle was located ventrolaterally to the PMC. This region was referred to as the PUSC or L-region. The PUSC was found to correspond to the nucleus locus subcoeruleus in rats, cats and dogs. Neurones from this region project directly to the Onuf's nucleus (Holstege *et al.*, 1986). Stimulation of the PUSC exerts a continuous excitatory effect on the Onuf's nucleus, thus resulting in urethral contraction and maintenance of urinary continence (Griffiths *et al.*, 1990).

1.4 Tracer studies using pseudorabies virus (PRV) – What do they reveal?

Transneuronal tracing with the Bartha Strain of the PRV has been used to identify the putative spinal and brain neurones that innervate the lower urinary tract. Injection of the PRV results in its transportation to a neuronal cell body via its axon, where it is replicated and dispersed throughout the cytoplasm and dendritic area. The PRV crosses synaptic gaps into axons that are only synaptically connected and thus its wide application in retrograde labelling.

Bladder injections of PRV displayed labelling in the medulla and pons, raphe magnus, raphe pallidus and A5. Heavy labelling was also observed in Barrington's nucleus following injection of PRV after bladder injections (Marson, 1997). Labelling in the spinal cord following bladder injections was observed in numerous cells located in the L6-S1, specifically the intermediolateral cell column (Marson, 1997) where majority of the parasympathetic preganglionic neurones and the dorsal gray commisure are located. Injections into the external urethral sphincter of rats resulted in the labelling of many regions in the brain (Nadelhaft & Vera, 1996). These labelled regions included serotonergic nuclei (raphe obscurus and raphe pallidus), noradrenergic nuclei (locus coeruleus and subcoeruleus), and to a lesser degree the pontine micturition center. Similarly, in cats, injections into the external urethral sphincter identified neurones in the raphe nuclei and the pontine micturition center. Areas of the spinal cord that were labelled following injections into the external urethral sphincter included the pudendal motorneurones in the dorsolateral nucleus of the L6 which is known to control external urethral sphincter activity. Also labelled were the contralateral and dorsomedial motorneurones (Marson, 1997).

1.5 Mechanisms involved in voiding

When the bladder is empty, parasympathetic preganglionic neurones are quiescent. Following urine accumulation, information is relayed to the sacral spinal cord via pelvic nerve Aδ-fibers where this information has both segmental and supraspinal actions (see Barrington, 1925, 1931). This ascending bladder sensory information is relayed to the PMC which switches off continence and initiates micturition. Information from the PMC is relayed through direct connections to the sacral parasympathetic preganglionic neurons (Blok & Holstege, 1997) as well as actions mediated by sacral spinal interneurones (Blok & Holstege, 1999; Shefyck, 2001) back to the bladder. For micturition to occur, the sympathetic efferent output to the bladder is depressed whilst sacral bladder parasympathetic preganglionic neurones are recruited (see de Groat *et al.*, 1999; McMahon & Morrison, 1982). Bladder contraction and urine expulsion through the urethra occurs following parasympathetic efferent output to the bladder smooth muscle and relaxation of the urethral sphincter and pelvic floor. During bladder filling and micturition, in rats and dogs (Bennett *et al.*, 1995; Kakizaki & de Groat, 1996; Kruse *et al.*, 1993; Maggi *et al.*, 1986), excitatory inputs mediated by somatic nerves exhibit external urethral sphincter activity which is thought to be necessary for efficient voiding as well as controlling alternate contractions and relaxations of the urethral outlet. In humans, external urethral sphincter activity is low during the early filling phase, rises with increases in bladder volume but is completely inhibited immediately prior to and during bladder emptying (guarding reflex; Brading, 1999). When the bladder volume reaches a critical threshold, the EUS relaxes, detrusor muscles contract and the bladder neck opens, thus resulting in voiding.

1.6 Clinical evidence for the relevance of the 5-HT_2 receptor to humans

A vast amount of research has been carried out to investigate the involvement of 5-HT_2 receptors in obesity and depression. Briefly, the 5-HT_{2C} receptor agonist mCPP has been vastly used in obesity studies where it was observed to reliably reduce food intake in lean and obese humans (see Halford *et al.*, 2007). Additionally, other 5-HT_{2C} receptor agonists such as Org 12962 (Organon), Ro 60-0175 (Roche and Vernalis), VER-3323 (Vernalis), BVT-933 (Biovitrum and GlaxoSmithKline) and YM348 (Yamanouchi Pharmaceuticals) have been investigated with some compounds passing into phase I of clinical trials (see Halford *et al.*, 2007). With respect to depression, selective serotonin reuptake inhibitors (SSRIs) are the most widely used drugs to treat depression due to their minimal side effects. A few antidepressants (nefazodone, trazodone and mirtazapine) act as antagonists at the 5-HT_{2A} receptor which is thought to be an important property that underlies the therapeutic properties of the drug. Indeed blockade of 5-HT_{2A} receptors is thought to affect the onset of the therapeutic actions of the (SSRIs), thus improving the drug effects (Caleda *et al.*, 2004). There is currently no evidence for the clinical use of drugs acting at the 5-HT_{2B} receptor to produce therapeutic effects in humans. Preclinical studies have also revealed a role for the 5-HT_2 receptor in other conditions such as sexual dysfunction, anxiety and schizophrenia amongst others.

1.7 5-HT/5-HT receptor involvement in the lower urinary tract

Currently, 14 different 5-HT receptor subtypes exist and these have been classified into seven different receptor families based on receptor homology, signalling pathway and operational characteristics (5-HT_{1-7}; see Hoyer *et al.*, 1994; Saxena, 1995).

Transneuronal tracer studies in rats using PRV, revealed labelling of the serotonergic nuclei following injections into the bladder and the external urethral sphincter. The labelled regions included the raphe magnus, raphe pallidus and raphe obscurus (Nadelhaft & Vera, 1996; Marson, 1997). Injections of PRV into the urethral smooth muscle also infected serotonergic regions, specifically the raphe magnus and periaqueductal gray (Vizzard *et al.*, 1995). Immunocytochemical studies in rats, cats and non-human primates also revealed that the lumbosacral sympathetic and parasympathetic autonomic nuclei, and the Onuf's nucleus in the lumbosacral spinal cord, received strong serotonergic inputs from the raphe nuclei (Skagerberg & Bjorklund, 1985; Kojima *et al.*, 1983; Rajaofetra *et al.*, 1992; Mizukawa, 1980; Bowker *et al.*, 1981). Additionally, it was also revealed that the urothelium of the urethra contained endocrine-like cells storing 5-HT (Hakanson *et al.*, 1974), and that 5-HT induced increases in urethral pressure (Creed & Tulloch, 1978).

5-HT has been shown to play an important role in the control of the lower urinary tract. Studies investigating supraspinal influences on bladder function found that activation of the raphe nuclei inhibited reflex bladder contractions, as well as the sacral efferent pathways to the bladder (see de Groat *et al.*, 1993; McMahon & Spillane, 1982). The mechanism proposed for these influences was that raphe neurones inhibited bladder activity through a spinobulbospinal negative-feedback circuit, where ascending sensory input from the bladder activated a descending inhibitory projection back to the sacral spinal cord (see Fraser & Chancellor, 2003). To elaborate further, the raphe neurones were proposed to influence bladder function by mediating an inhibitory control of sacral parasympathetic outflow and facilitatory control of the lumbar sympathetic outflow (possibly aiding in urine storage). What is

interesting however is that neuronal recording of single units in the raphe nucleus of the cat revealed that these neurones were activated by bladder distension (Lumb, 1986). This therefore raises the possibility that the raphe neurones may also be involved in a mechanism for voiding via excitatory influences.

Several pharmacological studies have tried to provide insight into the influences of 5-HT on bladder function. In cats, iontophoretic application of 5-HT to spinal autonomic neurones demonstrated prolonged activation of thoracic preganglionic neurones (de Groat & Ryall, 1967) and inhibition of firing of parasympathetic preganglionic neurones in the sacral spinal cord. Subsequent studies showed that reflex bladder activity and reflex bladder discharges on bladder parasympathetic nerves were depressed following intravenous administration of 5-hydroxytryptophan (5-HTP; a precursor for 5-HT), and tryptophan (Morgan *et al.*, 1979). Additionally, it has been revealed that 5-HT inhibits firing of spinal dorsal horn neurones evoked by stimulation of pelvic nerve afferents. It was also revealed that 5-HT receptors within the Onuf's nucleus caused contractions of the external urethral sphincter following electrical stimulation (see Fraser & Chancellor; 2003; Holstege *et al.*, 1986) and that 5-HT agonists enhanced external urethral sphincter reflexes which were depressed by 5-HT antagonists (Thor *et al.*, 1990; Downie & Bialik, 1988). From the above studies, it would appear that 5-HT is mainly inhibitory on bladder and urethral function, although an excitatory effect cannot be ruled out. An important question to ask therefore is whether supraspinal and spinal influences of 5-HT render a mechanism for urine storage/continence. This notion may however be reinforced by the fact that activation of 5-HT receptors facilitates a bladder-to-sympathetic reflex following bladder distension (Thor *et al.*, 1990; Morgan *et al.*, 1979), and this reflex is

clearly known to aid in maintaining urinary continence (de Groat & Lalley, 1972; de Groat & Theobald, 1976).

Outlined below is a detailed summary of distribution in the central nervous system (and peripheral tissues), receptor pharmacology, actions at the molecular level and functional characteristics of the 5-HT_{1A}, 5-HT_2, 5-HT_3, 5-HT_4, 5-ht_{5A}, 5-HT_6 and 5-HT_7 receptor families. The role of these receptors in micturition has also been described.

1.7.1 5-HT_{1A} receptor

To date, the 5-HT_{1A} receptor is the best characterised of the 5-HT receptors. This mainly emerged from early studies identifying the selective 5-HT_{1A} agonist, 8-OH-DPAT (Gozlan *et al.*, 1983) as well as from receptor cloning (Kobilka *et al.*, 1987; Albert *et al.*, 1990).

1.7.1.1 Receptor distribution

Receptor autoradiography studies using various ligands such as [^{3}H]-5-HT, [^{3}H]-8-OH-DPAT and more recently [^{3}H]-WAY 100635 (Pazos & Palacios, 1985; Hoyer *et al.*, 1986) identified 5-HT_{1A} receptors in the limbic brain areas which included the hippocampus, lateral septum, cortical areas (cingulate and entorhinal cortex) and the mesenphalic raphe nuclei (dorsal and median raphe nuclei; see Barnes & Sharp, 1999). At the sub-cellular level, 5-HT_{1A} receptors were found to be located postsynaptic to 5-HT neurones in forebrain regions, as well as on the soma and dendrites in the mesenphalic and medullary raphe nuclei (see Miquel *et al.*, 1991, 1992). Localization of the 5-HT_{1A} receptor to the somatodendritic region (presynaptic) of neurones isolated from the raphe nuclei have been shown to act as autoreceptors which inhibit cell firing. Furthermore, in situ hybridisation and immunocytochemical studies found 5-HT_{1A} receptors in pyramidal neurones of the cortex and hippocampus and also hippocampal granular neurones (Pompeiano *et al.*, 1992; Burnet *et al.*, 1995). The receptors in the hippocampus were found to be heteroreceptors with a postsynaptic location (Palacios *et al.*, 1991; Ghavami *et al.*, 1999; Riad *et al.*, 2000). Additionally, the 5-HT_{1A} receptor is also expressed in 5-HT-containing neurones in the raphe nuclei, cholinergic neurones in the septum and possibly glutamatergic neurones in the cortex and hippocampus (Kia *et al.*, 1996).

Peripherally, mRNA coding for the 5-HT_{1A} receptor has been detected in the lymphatic tissue, gut, muscle and kidney (Kobilka *et al.*, 1987; Albert *et al.*, 1990; Chalmers & Watson, 1991).

1.7.1.2 Receptor pharmacology

Several agonists have been developed to act at the 5-HT_{1A} receptor and these include buspirone, ipsaspirone, gepirone, tandospirone, BMY 7378, and NAN-190 (Zifa & Fillon, 1992). These ligands were however found to act as partial agonists in animal models even though they acted as full agonists in recombinant systems. Breakthrough in 5-HT_{1A} studies came from the development of the highly selective prototypical 5-HT_{1A} receptor agonist 8-OH-DPAT (K_d = 0.23-1.8 nM) which displays high potency *in vivo*, and continues to be the agonist of choice in 5-HT_{1A} receptor studies. Non-selective antagonists propanolol, spiperone and pindolol were the only commercially available antagonist tools used to investigate the 5-HT_{1A} receptor for a long time. A number of 5-HT_{1A} 'silent' receptor antagonists have now been developed and these include WAY 100635, WAY 100135 and NAD-299. The term 'silent' antagonist has been used for such compounds in order to distinguish them from several 5-HT_{1A} receptor partial agonists that were initially designated 'antagonists'. Of the three compounds, WAY 100635 has been found to be the most potent antagonist of the group (Fletcher *et al.*, 1996; Johansson *et al.*, 1997). An in depth study carried out by Fletcher and colleagues (1996) investigating the electrophysiological, biochemical, neurohormonal and behavioural properties of WAY 100635 further demonstrated the potent selectivity properties of the antagonist at the 5-HT_{1A} receptor. Briefly, WAY 100635 was found to have an IC_{50} of 1.35 nM, with greater than 100-fold selectivity for the 5-HT_{1A} site relative to a range of other receptors (Fletcher *et*

al., 1996). In mice, intravenous administration of [^{3}H]WAY-100635 was shown to bind selectively to brain 5-HT_{1A} receptors *in vivo*. Electrophysiological studies demonstrated blockade of the effects of agonists by WAY 100635, at both the postsynaptic 5-HT_{1A} receptor in the hippocampus, and the somatodendritic 5-HT_{1A} receptor located on raphe 5-HT neurones. Furthermore, WAY 100635 was found to dose-dependently block the inhibitory effects of 8-OH-DPAT on raphe 5-HT neuronal firing.

1.7.1.3 Signal transduction

The 5-HT_{1A} receptor couples negatively via G proteins (α_i) to adenylate cyclase in both rat and guinea pig hippocampal cell lines expressing the cloned version of the receptor (see Boess & Martin, 1994; Sadou & Hen, 1994). Other effects involving the 5-HT_{1A} receptor in transfected cell-lines include decreased or increased intracellular Ca^{2+} and activation of phospholipase C (see Boess & Martin, 1994). However, there is no solid evidence for such coupling in brain tissue.

1.7.1.4 Physiological functions

5-HT_{1A} receptors have been implicated in mediating various behavioural and physiological effects such as hyperphagia, hypothermia, altered sexual activity, a tail flick response (see Green & Grahame-Smith, 1976; Millan *et al.*, 1991; Lucki, 1992) and bladder function. Additionally, there is a vast amount of basic and clinical data in the literature surmising the anxiolytic and antidepressant activity of 5-HT_{1A} receptor agonists (Traber & Glaser, 1987; Charney *et al.*, 1990; Handley, 1995).

1.7.1.5 5-HT_{1A} receptors – excitatory or inhibitory?

5-HT_{1A} receptor activation causes inhibition characterised by neuronal hyperpolarisation, and this is mediated via G-protein-coupled opening of K^+ channels (see Nichol *et al.*, 1990; Aghajanian, 1995). Ionotophoretic administration of 5-HT_{1A} receptor agonists and 5-HT have been shown to inhibit neuronal activity in rat hippocampus, frontal cortex and other brain areas (Sprouse & Aghajanian, 1988; Ashby *et al.*, 1994 a,b), and these effects have been blocked by both non-selective (spiperone and methiothepin) and selective (WAY 100635) 5-HT_{1A} receptor antagonists. Similar effects have also been observed in 5-HT containing neurones in the raphe nuclei following administration of 5-HT_{1A} receptor agonists and antagonists (Sprouse & Aghajanian, 1988).

1.7.1.6 5-HT_{1A} receptors and micturition

NAN 190 and BMY 7378 have been shown to demonstrate antagonist-like activity in measures of postsynaptic 5-HT_{1A} receptor activation and agonist-like activity when examined at the somatodendritic 5-HT_{1A} autoreceptors (Hjorth & Sharp, 1990; Sharp *et al.*, 1990). By acting as partial 5-HT_{1A} receptor agonists at the somatodendritic 5-HT_{1A} autoreceptors, NAN 190 and BMY 7378 were shown to inhibit the firing of raphe neurons in rats (Gobert *et al.*, 1995) and cats (Fornal *et al.*, 1994), thus leading to inhibition of 5-HT turnover in different brain areas and the spinal cord. It was proposed that decreases in spinal 5-HT inhibited the endogenous spinobulbospinal negative feedback mechanism, thus leading to excitatory effects on the micturition reflex. Similarly, full 5-HT_{1A} receptor agonist 8-OH-DPAT and strong partial agonist Rec 0/0249 also inhibited raphe neuronal firing, leading to increased frequency of voiding contractions (Lecci *et al.*, 1992) and decreased bladder capacity (Testa *et al.*,

1999). Conversely, WAY 100635 was observed to increase the firing rate of raphe nuclei cells of rats *in vitro* (Corradetti *et al.*, 1996) as well as in cats and guinea pigs *in vivo* (Fornal *et al.*, 1996), thus leading to an increase in spinal 5-HT which in turn inhibited the micturition reflex.

Several pharmacological studies have provided evidence for the involvement of 5-HT_{1A} receptor agonists and antagonists in the control of the lower urinary tract function. Lecci *et al* (1992) demonstrated that intravenous (i.v.), intracerebroventricular (i.c.v.) and intrathecal (i.t.) administration of the selective 5-HT_{1A} receptor agonist 8-OH-DPAT increased the frequency of bladder contractions in both normal and spinal-cord injured (SCI) rats, thus suggesting the 5-HT_{1A} receptor was excitatory in micturition. Additionally, bladder capacity was found to be reduced following i.v. administration of 8-OH-DPAT in conscious rats (Testa *et al.*, 1999). Another study that corroborated with the above findings was from Conley *et al* (2001) where following administration of 8-OH-DPAT and BMY 7378 (also an α_{1D}-adrenoceptor antagonist), a significant decrease in bladder pressure and volume threshold in anaesthetized male rats was observed. The effects caused by BMY 7378 were confirmed as 5-HT_{1A} receptor mediated rather than α_{1D} receptor mediated, as a dose of WAY 100635 found to be ineffective on the micturition reflex blocked the effects of BMY 7378. Interestingly, Conley and colleagues found that BMY 7378 and WAY 100635 reduced baseline urethral pressure and inhibited reflex urethral contractions. As BMY 7378 possesses selectivity for α_1-adrenoceptor subtypes, it was concluded these effects were mediated via α_{1A}- and α_{1D}-adrenoceptors which is the next receptor family that WAY 100635 has affinity for (Forster *et al.*, 1995). Gu and colleagues (2004) investigated the effects of i.v. administration of 8-OH-DPAT

on bladder capacity and EUS-EMG activity in chronic spinal cord injured (SCI) and intact cats. Their results from this study demonstrated that following administration of 8-OH-DPAT in intact cats, the effects on bladder capacity were variable, even though they tended towards an increase, with no significant effects observed on EUS-EMG activity. The group postulated that the inconsistencies observed on bladder capacity may have been due to the dual role of the 5-HT_{1A} receptors as both presynaptic autoreceptors and postsynaptic receptors. Interestingly, i.v. administration of 8-OH-DPAT in SCI cats significantly increased volume threshold, bladder capacity and residual volume and these effects were reversed by the selective 5-HT_{1A} receptor antagonist WAY 100635 thus confirming that 5-HT_{1A} receptors were involved in mediating the effects observed. From these results, the group provided evidence for a possible beneficial role of the 5-HT_{1A} receptor in suppressing bladder hyperreflexia.

I.v. administration of spiroxitane, NAN 190 and high doses of buspirone, which all possess antagonistic-like properties at the 5-HT_{1A} receptor, were found to inhibit isovolumetric bladder contractions in a dose dependent manner (Lecci *et al.*, 1992). However, dual/multiple actions of these compounds makes it difficult to draw solid conclusions regarding the physiological role of 5-HT_{1A} receptors in micturition. This issue has been overcome through the use of the selective 5-HT_{1A} receptor antagonist WAY 100635 which acts as an antagonist at both the pre- and postsynaptic level, unlike other 5-HT_{1A} receptor antagonists. Support for a physiological role of 5-HT_{1A} receptor antagonists arises from isovolumetric experiments carried out by Kakizaki and colleagues (2001) where the group showed dose dependent inhibition of rhythmic bladder contractions following i.t. (L6-S1) administration of WAY 100635. The

group also found that i.t. administration of WAY 100635 at the thoracic and cervical levels of the spinal cord did not have any effect on rhythmic bladder contractions, thus suggesting that spinal 5-HT_{1A} receptors at the L6-S1 level are involved in bladder function. Systemic (i.v.) administration of WAY 100635 also produced significant inhibition of rhythmic bladder contractions. In conscious rats, i.v. administration of WAY 100635 has also been shown to increase bladder capacity (Testa *et al.*, 1999). More evidence for an inhibitory action of 5-HT_{1A} receptor antagonists on the micturition reflex was provided by i.v. injections of another structurally related antagonist to WAY 100635, p-MPPI, which was shown to inhibit the regular isovolumetric bladder contractions (Testa *et al.*, 2001). Interestingly, the inhibitory effect of i.v WAY 100635 was antagonized by pretreatment with mesulergine, a 5-HT_2 receptor antagonist in anaesthetized rats whereas the 'partial agonist' NAN-190 was unaffected (Testa *et al.*, 1999). Similar results have been obtained following pre-treatment of WAY 100635 with the 5-HT_{2A} receptor antagonist ketanserin where the rats were devoid of inhibitory effects, therefore suggesting the involvement of 5-HT_2 receptors. De Groat (2002) proposed a supraspinal 5-HT_{1A} autoreceptor pathway involving 5-HT_2 receptors as a possible explanation for the blockade of the effects of WAY 100635 on the micturition reflex (see section 1.7).

1.7.2 5-HT_2 receptor

The 5-HT_2 receptor family comprises three receptor subtypes, 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}, which have been reported to play a major role in a range of central nervous system functions including anxiety, depression, migraine, sleep, satiety and schizophrenia (Baxter *et al.*, 1994). All three receptor subtypes are classified into the same family as they couple to identical signalling pathways, possess similar pharmacological profiles as well as a similarity in (gene) structure (Humphrey *et al.*, 1993). The 5-HT_2 receptors are associated with the Gq family of G-proteins and upon activation, stimulate phospholipase C to increase phosphoinositide metabolism, thus causing a transient rise in intracellular free calcium (Martin & Humphrey, 1994; Roth *et al.*, 1998; Porter *et al.*, 1999).

1.7.2.1 5-HT_{2A} receptor

1.7.2.1.1 Receptor distribution

The 5-HT_{2A} receptor has been cloned from rat (Pritchett *et al.*, 1988), human (Stam *et al.*, 1992), mouse (Foguet *et al.*, 1992; Yang *et al.*, 1992), hamster (Chambard *et al.*, 1990; Van Obberghen-Schilling *et al.*, 1991), pig (Johnson *et al.*, 1995) and rhesus monkey (Johnson *et al.*, 1995).

Receptor autoradiography, in situ hybridisation and immunocytochemistry have enabled the mapping of 5-HT_{2A} receptors in the CNS. 5-HT_{2A} receptor distribution has been found in many cortical areas (neocortex, entorhinal and pyriform cortex, and claustrum), caudate nucleus, nucleus accumbens, olfactory tubercle and hippocampus of various species (Pazos *et al.*, 1985, 1987; López-Giménez *et al.*, 1997). Regarding location in the brain, it has been reported that the 5-HT_{2A} receptor-like

immunoreactivity or 5-HT_{2A} receptor mRNA has been found in neurones such as local GABAergic interneurons, cortical pyramidal neurones (Burnet *et al.*, 1995) and cholinergic neurones in the basal forebrain and the brainstem (Morilak *et al.*, 1993). 5-HT_{2A} receptor mRNA has also been detected in the spinal cord of the rat, cat, monkey and human tissue using polymerase chain reaction with southern hybridization analysis (Helton *et al.*, 1994). Additionally, molecular studies have also revealed the presence of 5-HT_{2A} receptors in the cardiovascular system of several species. In humans for example, 5-HT_{2A} receptors have been observed to be widespread on arterial smooth muscles (Ullmer *et al.*, 1995).

A postsynaptic location has been implicated for the 5-HT_{2A} receptor. This is due to the fact that the 5-HT_{2A} binding sites, 5-HT_{2A} mRNA and 5-HT_{2A} receptor-like immunoreactivity, which are all distributed within the same region (Mengod *et al.*, 1990; Morilak *et al.*, 1993, 1994; Pompeiano *et al.*, 1994; Burnet *et al.*, 1995), are located in the region where the receptors are present. However, immunocytochemical studies also deduced a presynaptic location of 5-HT_{2A} receptors on nerve terminals (Jakab & Goldman-Rakic, 1998).

1.7.2.1.2 Receptor pharmacology

Initially, discrimination of the 5-HT_{2A} receptor subtype from either 5-HT_{2B} or 5-HT_{2C} receptors proved to be a challenge due to the lack of selective agonists and antagonists for the receptor. Antagonists such as ketanserin and spiperone with 40-fold and over 100-fold selectivity for 5-HT_{2A} receptors (over 5-HT_{2B} or 5-HT_{2C} respectively; Jerman *et al.*, 2001) were initially used to make pharmacological distinctions between the 5-HT_2 receptor family. The development of MDL 100907 (Ki = 0.78 nM at the

human 5-HT_{2A} receptor; Gleason *et al.*, 2001) a potent antagonist of the 5-HT_{2A} receptor with a low affinity for 5-HT_{2C} and other receptors, enabled further discrimination between the 5-HT_2 family. In addition to the above, the development of selective 5-$HT_{2B/2C}$ receptor antagonists SB 200646A and SB 206553, 5-HT_{2B} receptor antagonist SB 204741 and 5-HT_{2C} receptor antagonists RS 102221 and SB 242084 have also made it possible to make further discriminations between the 5-HT_2 receptor family. However, even though progress has been made regarding development of selective 5-HT_{2A} receptor antagonists, at present there are still no suitable commercially available 5-HT_{2A} receptor agonists. Therefore compounds such as DOI and its structural analogues (DOB and DOM; Newton *et al.*, 1996; Porter *et al.*, 1999) and quipazine have been labelled as 5-HT_{2A} 'preferring' receptor agonists due to their appreciable affinity for the 5-HT_{2A} receptor.

Interestingly, a species variation was found to exist when studies comparing the pharmacological profile of the rat and human 5-HT_{2A} receptor were carried out. It was found that the rat receptor had a much higher affinity for mesulergine (5-HT_{2C} receptor antagonist) than the human receptor (Ki = 5nM in rat vs Ki =150 nM in human), even though the human 5-HT_{2A} receptor is highly homologous to that of the rat receptor (Kao *et al.*, 1992; Johnson *et al.*, 1994).

1.7.2.1.3 Signal transduction

When stimulated, the 5-HT_{2A} receptor was found to activate phospholipase C in both heterologous expression systems (Pritchett *et al.*, 1988; Stam *et al.*, 1992) and brain tissue (Conn & Sanders-Bush, 1984; Godfrey *et al.*, 1988) through a G-protein coupling mechanism. An example of this was shown in Xenopus oocytes or HEK293

where expression of the 5-HT_{2A} receptor led to activation of phospholipase C, Ca^{2+} mobilisation, or activation of Ca^{2+}-induced Cl^- current. However, additional signalling pathways have been reported for endogenously expressed 5-HT_{2A} receptors. These include inhibition or activation of adenylyl cyclase (Garnovskaya *et al.*, 1995; Berg *et al.*, 1994 respectively) or activation of phospholipase A_2 (Felder *et al.*, 1990).

An interesting phenomenon reported for the 5-HT_{2A} receptor in some *in vivo* and *in vitro* models is a down-regulation of the receptor following chronic exposure to antagonists such as mianserin, spiperone and mesulergine (Sanders-Bush, 1990; Roth & Ciaranello, 1991; Grotewiel & Sanders-Bush, 1994). It has been proposed that this down-regulation may possibly be a contributory factor towards the action of some ligands as inverse agonists (see Barnes & Sharp, 1999).

1.7.2.1.4 Physiological functions

The involvement of the 5-HT_{2A} receptor subtype in both peripheral and central functions has been widely investigated to date. The 5-HT_{2A} receptor has therefore been implicated in various functions such as smooth muscle contraction, platelet aggregation, control of hormone or transmitter release, control of sexual activity, sleep regulation, motor behaviour, and psychiatric disorders such as epilepsy, migraine, anxiety, depression, schizophrenia and hallucinations (see Gerhardt & Heerikhuizen, 1997). However, it should be noted that the functions listed above are not mediated exclusively by the 5-HT_{2A} receptors as the agonists used to elucidate the above functions are only partially selective for the 5-HT_{2A} receptor, possessing selectivity for other receptors.

1.7.2.2 5-HT_{2B} receptor

1.7.2.2.1 Receptor distribution

The 5-HT_{2B} receptor (originally known as 5-HT_{2F} receptor) was originally found to be located in the stomach fundus smooth muscle of the adult rat (Foguet *et al.*, 1992; Kursar *et al.*, 1992) and was thus implicated in contraction of this smooth muscle. Although previously reported as present in the human brain but not in the rat brain (Kursar *et al.*, 1994; Schmuck *et al.*, 1994), Duxon *et al* (1997) showed the presence of 5-HT_{2B} receptors in the rat brain, more specifically in the cerebellum, cortex, hippocampus and amygdala. Similar locations were found to express 5-HT_{2B} receptors in the mouse brain (Choi & Maroteaux, 1996). 5-HT_{2B} receptor mRNA was also identified in the spinal cord of the rat, cat, monkey and humans (Helton *et al.*, 1994). 5-HT_{2B} receptors have also been found in a variety of peripheral tissues such as the adult gut and cardiovascular system (Loric *et al.*, 1992), which include the stomach, intestine and pulmonary smooth muscle, pulmonary and vascular endothelial cells and cardiomyocytes in mouse (Choi & Maroteaux, 1996), human (Choi *et al.*, 1994; Schmuk *et al.*, 1994; Bonhaus *et al.*, 1995) and rat (Foguet *et al.*, 1992; Kursar *et al.*, 1992; Baxter *et al.*, 1994; Ullmer *et al.*, 1995).

1.7.2.2.2 Receptor pharmacology

The pharmacological profile of the 5-HT_{2B} receptor has been elucidated using displacement of [^{3}H] 5-HT (Foguet *et al.*, 1992; Bonhaus *et al.*, 1995) or [^{125}I]DOI (Loric *et al.*, 1992; Choi *et al.*, 1994) binding. Of all the three 5-HT_2 receptor subtypes, the 5-HT_{2B} receptor has been the easiest receptor to discriminate from the other two due to its lower affinity for mianserin and ritanserin (non selective 5-HT_2 receptor antagonists) and higher affinity for 5-HT and yohimbine (α_2-adrenergic

antagonist). Furthermore, the use of SB 204741 has shown 20-60 fold selectivity for the 5-HT_{2B} receptor relative to 5-HT_{2A}, 5-HT_{2C} or other receptors at which it has been tested (Bonhaus *et al.*, 1995; see Baxter, 1996). RS 127445 has been described as the most selective commercially available 5-HT_{2B} receptor antagonist as it has reduced selectivity for the 5-HT_{2A} receptor and greater than 400-fold selectivity over the 5-HT_{2C} receptors. To date, the only agonist that has been described as possessing selectivity for the 5-HT_{2B} receptor is BW 723C86 as it has about 10-fold selectivity for the 5-HT_{2B} receptor when compared to either the 5-HT_{2A} or 5-HT_{2C} receptor.

Interestingly, although both the rat and human 5-HT_{2B} receptors display a similar pharmacological profile, there are some differences where for example affinity for the 5-HT_{2A} receptor antagonists ketanserin and spiperone is observed to be much higher for the human than the rat receptor. Similarly, a species difference exists between the mouse 5-HT_{2B} receptor compared to the rat or human 5-HT_{2B} receptors where the mouse 5-HT_{2B} receptor has a lower affinity for 5-HT and a higher affinity for spiperone (Bonhaus *et al.*, 1995; Wainscott *et al.*, 1996).

1.7.2.2.3 Signal transduction

When expressed in Xenopus oocytes, the rat 5-HT_{2B} receptor activation opens up Ca^{2+}-activated chloride channels due to the activation of phospholipase C (Foguet *et al.*, 1992). This response is less pronounced and thus distinct from the other 5-HT_2 receptor subtypes. An additional signalling pathway, cAMP production, has been observed following expression of 5-HT_{2B} receptors in AV12 cells (Lucaites *et al.*, 1996).

1.7.2.2.4 Physiological functions

5-HT_{2B} receptors have primarily been shown to contract stomach and intestinal smooth muscle (Vane, 1959; Baxter *et al.*, 1994; Borman & Burleigh, 1995). However, several studies have shown that 5-HT_{2B} receptors, relax the vascular endothelium through stimulation of nitric oxide release (Glusa & Richter, 1993; Bodelsson *et al.*, 1993; Ellis *et al.*, 1995). In obesity, systemic administration of 5-HT_{2B} receptor agonist BW 723C86 was found to dose-dependently increase food intake in free feeding rats (Kennett *et al.*, 1997). This increase was suggested to be as a result of an increase in gastric emptying due to the primary role played by 5-HT_{2B} receptors in contracting smooth and intestinal smooth muscle. Activation of 5-HT_{2B} receptors has also shown an association of the receptors with pulmonary hypertension (Launay *et al.*, 2002) and irritable bowel syndrome (Borman *et al.*, 2002).

1.7.2.3 5-HT_{2C} receptor

1.7.2.3.1 Receptor distribution

Pazos *et al* (1984) found the 5- HT_{2C} receptor to be highly expressed in the choroid plexus of several mammalian species (Pazos *et al.*, 1984). Subsequently, regional distribution studies identified 5- HT_{2C} receptors in other parts of the rat, human and monkey brain. In the rat, 5-HT_{2C} mRNA was found in the hippocampus, cerebral cortex, striatum and substantia nigra, whereas in humans 5-HT_{2C} mRNA was found in the cerebral cortex, substantia nigra, cerebellum and amygdala (Abramowski *et al.*, 1995). In the monkey brain, using in situ hybridization, López-Giménez *et al* (2001), detected 5-HT_{2C} mRNA in layer V of the cortex, nucleus accumbens, anterior caudate and putamen, septal nuclei, amygdala, as well as several thalamic, midbrain and brainstem nuclei. In the spinal cord of the rat, monkey and humans, 5-HT_{2C} receptor

mRNA was detected using polymerase chain reaction with southern hybridization analysis (Helton *et al.*, 1994). Interestingly, even though 5-HT_{2C} mRNA is widely expressed in the central nervous system, there is low to absent expression in the peripheral tissues.

5-HT_{2C} receptors are mainly found postsynaptically (Palacios *et al.*, 1991; Cornea-Hebert *et al.*, 1999; Verge & Calas, 2000), but there is the possibility of a presynaptic location.

1.7.2.3.2 Receptor pharmacology

It is difficult to make a sufficient discrimination of 5-HT_{2C} receptors from 5-HT_{2A} and 5-HT_{2B} receptors due to a lack of selective ligands. This means that the selectivity for most of the 5-HT_{2C} receptor agonists is deduced from their reduced intrinsic efficacy at 5-HT_{2A} and 5-HT_{2B} receptors rather than their binding selectivity. For many years, mCPP has been used as the typical 5-HT_{2C} receptor agonist as it has a greater affinity for cloned human 5-HT_{2C} receptors (K_i = 16 nM) over the 5-HT_{2A} (K_i = 85 nM) and 5-HT_{2B} (K_i = 40 nM; Kimura *et al.*, 2004). Over the years, other commercially available 5-HT_{2C} receptor agonists have emerged and these include Ro 60-0175 (K_i = 1 nM) which is more selective over the 5-HT_{2A} receptor (K_i = 32 nM; Hemrick-Luecke & Evans, 2002) in cloned rat binding studies. Other reportedly selective 5-HT_{2C} receptor agonists include WAY 161503 (K_i = 3 nM), WAY 163909 (K_i = 10 nM) which is more selective than 5-HT_{2A} (K_i = 212 nM) and 5-HT_{2B} (K_i = 485 nM; Dunlop *et al.*, 2005) receptors and YM-348 (Ki = 0.89 nM) which is more selective at the 5-HT_{2C} receptor than the 5-HT_{2A} (K_i = 13 nM) and 5-HT_{2B} (K_i = 2.5 nM; Kimura *et al.*, 2004) receptors. Antagonist studies have also been used to further distinguish 5-HT_{2C} receptors from either 5-$HT_{2A/2B}$ receptors. The development of selective 5-

HT_{2C} receptor antagonists SB 242084 and RS 102221, which are at least two orders of magnitude more selective for the 5-HT_{2C} receptor versus the 5-HT_{2B}, 5-HT_{2A} receptors and other binding sites (Bonhaus *et al.*, 1997; Kennett *et al.*, 1997) have led to a better understanding of the pharmacology of 5-HT_{2C} receptors.

1.7.2.3.3 Signal transduction

Phospholipase C activity is increased following activation of the 5-HT_{2C} receptors in the choroid plexus of various species (Sanders-Bush *et al.*, 1988). This activity is mediated via a G-protein coupled mechanism (see Boess & Martin, 1994). It has also been suggested that in addition to activating phospholipase C, 5-HT_{2C} receptors may also trigger cGMP in the choroid plexus (Kaufman *et al.*, 1995).

Similar to 5-HT_{2A} receptors, continuous exposure of 5-HT_{2C} receptors to both agonists and antagonists down-regulates the receptor (Barker *et al.*, 1994; Labrecque *et al.*, 1995) and this is thought to contribute to the inverse agonist properties observed for certain 5-HT_{2C} agonists (e.g. mCPP which has inverse agonistic properties at the 5-HT_{2A} receptor).

1.7.2.3.4 Physiological functions

Activation of 5-HT_{2C} receptors has been reported to mediate various effects, including hypophagia, hypolocomotion, anxiety, hyperthermia, induction of penile erections (Kahn & Wetzler, 1991) and bladder function (Steers & de Groat, 1989). Most of these correlations have been based on behavioural results obtained using non-selective 5-HT_{2C} agonists such as mCPP, TFMPP, MK212 and antagonists such as ritanserin and mianserin. In addition to pharmacological studies, knockout mice have been used

to model obesity, where the mice lacking the 5-HT_{2C} receptor develop late-onset obesity (Tecott *et al.*, 1995) with no reversal properties observed following administration of mCPP, thus further elucidating the role of the 5-HT_{2C} receptor subtype.

1.7.2.4 5-HT_2 receptors – excitatory or inhibitory?

The role of 5-HT_2 receptors in the control of neuronal firing activity in the central nervous system is fairly controversial as conflicting results have been obtained following electrophysiological studies carried out *in vitro* and *in vivo*, as described below.

1.7.2.4.1 Excitatory effects

Several studies have implicated a direct excitatory effect of 5-HT_2 receptors on the central nervous system. In the brain, 5-HT_2 receptor activation was found to mediate neuronal excitation characterised by depolarisation, increased excitability of the neurones and neurotransmitter release. Evidence for this comes from several electrophysiological recordings carried out *in vivo* and *in vitro*, on various brain regions, where the effects of agonists and antagonists on these recordings were observed. 5-HT_{2A} receptor-mediated excitation in the cortex has also been observed (Sheldon & Aghajanian, 1991; Marek & Aghajanian, 1995) following intracellular recordings of interneurones in slices. This excitatory effect was concluded to occur as a result of reducing outward potassium currents, and was blocked by selective 5-HT_{2A} receptor antagonist MDL 100907. Depolarisations, thought to be 5-HT_{2A} receptor mediated have also been detected in slice preparations of the nucleus accumbens (North & Uchimura, 1989), neocortex (Aghajanian & Marek, 1997) and the dentate

gyrus (Piguet & Galvan, 1994). It has also been observed that glutamatergic pyramidal neurones in the rat cortex are depolarised following activation of 5-HT_{2C} receptors (Sheldon & Aghajanian, 1991) in *in vitro* electrophysiological studies.

Raphe spinal neurones are known to be the main source of 5-HT in the spinal cord (Schmidt & Jordan 2000) and they project extensively to most cell types in the spinal cord including motorneurones (Jacobs & Azmitia 1992; Kiehn *et al.* 1992). An excitatory effect has been observed on spinal cord neurones following activation of 5-HT_2 receptors. These receptors were found to cause depolarisations of dorsal horn neurones and motorneurones in the frog (Tan & Miletic, 1992; Holohean *et al.*, 1990) and these depolarisations were blocked by the 5-HT_2 receptor antagonists ketanserin, methylsergide and mianserin. Hori and colleagues (1996) observed long-lasting potentiations of excitatory postsynaptic currents (EPSC) amplitude and miniature EPSC frequency following administration of DOI in whole-cell recordings from superficial dorsal horn neurones in neonatal rat spinal cord slices. The group concluded that the above effects on the dorsal horn neurones were possibly mediated by 5-HT_2 receptors.

1.7.2.4.2 Inhibitory effects

Inhibitory effects of 5-HT_2 receptors on neuronal firing have been recorded in the rat brain, following application of 5-HT_2 receptor agonists (DOI and mCPP) by microiontophoresis onto the medial prefrontal cortex and the orbito-frontal cortex (Bergqvist *et al.*, 1999). However, this study ruled out the possibility that this inhibitory effect on firing was indirectly mediated by excitatory 5-HT_2 neurones on

GABA interneurons as bicuculline (GABA antagonist) and no effect on the inhibitory action of DOI and mCPP.

An electrophysiological study carried out on motorneurone slices from neonatal rats reported that 5-HT_2 receptors were inhibitory, as they caused potassium-mediated hyperpolarization (Wang & Dun, 1990). Additionally, the same study found that 5-HT via 5-HT_2 neurones indirectly affected the activity of motorneurones by activating inhibitory interneurones.

1.7.2.5 5-HT_2 receptors and micturition

Both *in vitro* and *in vivo* pharmacological studies have implicated the involvement of the 5-HT_{2A} and 5-HT_{2C} receptor subtypes in the control of the lower urinary tract in humans and animals. These receptors have been found to function as either excitatory or inhibitory on the bladder or urethra.

Serotonin was found to produce dose dependent contractions on isolated rat bladder muscle strips (Kim *et al.*, 2002). The 5-HT_{2A} receptor antagonist ketanserin, almost abolished the facilitatory effects of serotonin in the rat bladder muscles whereas propranolol, a non-selective beta blocker and 5-HT_1 receptor antagonist partially blocked the effects of serotonin on bladder contractility. Tropisetron, a 5-HT_3 receptor antagonist failed to inhibit the facilitatory effects of serotonin. These results demonstrated that the 5-HT_2 receptor (specifically 5-HT_{2A} receptor) was mainly responsible for serotonin-induced contraction of the detrusor, whereas the 5-HT_1 receptor was only partially responsible.

The effects of 5-HT_{2A} receptor agonist DOI on pudendal nerve reflexes in the anaesthetised cat was investigated by Danuser and Thor (1996). Pudendal nerves were isolated from both female and male cats where some had undergone a T12-T13 laminectomy. All the cats were pre-treated with the long lasting peripheral restricting 5-HT_2 receptor antagonist xylamidine, in order to eliminate any peripheral effects of DOI on the bladder or vascular smooth muscle. Intravenous administration of DOI (0.01-3 mg kg^{-1}) was found to dose dependently increase pudendal reflexes in acute spinal cats, but not spinally-intact cats. The group concluded that 5-HT_2 receptors facilitated pudendal reflexes through a facilitatory effect mediated in the spinal cord of acute spinal cats and these effects were counteracted by inhibitory supraspinal 5-HT_2 receptors as observed in the spinally-intact cats. In the same study, Danuser and Thor (1996) also investigated the effects of DOI on pelvic and hypogastric nerves where surprisingly DOI produced no significant effects on the pelvic to hypogastric reflex in either spinally-intact or acute spinal cats, thus excluding the involvement of 5-HT/5-HT_2 receptors in bladder contraction of the cat. The findings from this study are interesting as it has been documented elsewhere in the literature that 5-HT/5-HT_2 receptor is able to contract the urinary bladder from cat (Saxena *et al.*, 1985), guinea pig (Ambache & Zar, 1970) and rabbit (Longhurst *et al.*, 1984). In the dog, bladder contraction was observed following administration of α-methyl serotonin, an agonist with selectivity for the 5-HT_2 receptor and its effects were blocked by LY53857 (Cohen, 1990). In humans, the role of 5-HT_2 receptors in the lower urinary tract has been demonstrated from studies where the 5-HT_{2A} receptor antagonist ketanserin was found to cause significant decreases in urethral pressure with no significant changes on bladder function (Delaere, 1987). It was concluded that ketanserin would be useful

in mediating bladder emptying due to a reduction in urethral resistance in patients with outflow obstruction due to prostatic enlargement.

The involvement of 5-HT_{2C} receptors in the control of micturition was first demonstrated by Steers and de Groat (1989) with the findings confirmed by Guarneri *et al* (1996). In the study, intravenous administration of mCPP (100 μg kg^{-1}) inhibited rhythmic bladder contraction. Interestingly, following abolishment of somatic muscle contractions by administering the neuromuscular blocker pancuronium, mCPP did not inhibit bladder activity, thus suggesting a somatic influence on the actions of mCPP on bladder activity. Other 5-HT_{2C} receptor agonists TFMPP and MK212 were also found to depress reflex bladder activity. Guarneri and colleagues (1996) also demonstrated blockade of the inhibitory effect of mCPP on bladder activity by the non-selective 5-HT_2 receptor antagonists mesulergine and metergoline. A mechanism proposed for these inhibitory actions of 5-HT_{2C} receptors on micturition is that the raphe input to the spinal cord activates segmental inhibitory interneurons via 5-HT_{2C} receptors which in turn suppress the parasympathetic preganglionic neurones innervating the bladder. The effects of intravenous administration of 5-HT_2 receptor antagonists on the micturition reflex were investigated by Testa *et al* (2001). Mesulergine, ketanserin and SB 242084 had no effects on isovolumetric bladder contractions even at the highest dose of 1 mg kg^{-1}. However, when compared to basal values, administration of the low dose of mesulergine (10 μg kg^{-1}) and ketanserin (100 μg kg^{-1}) appeared to decrease the frequency of isovolumetric bladder contractions, although no significance was observed when comparisons were made with pre-treatment. Furthermore, it was reported that in some animals, mesulergine and SB 242084 showed a tendency to increase the frequency of bladder contraction. Espey

and Downie (1995) also reported that i.t. methylsergide (a partially selective 5-$HT_{2A/2C}$ receptor antagonist) induced reductions in the volumes at which micturition occurred in conscious spinally intact cats. Due to the variable results obtained from the antagonist data described above, the use of 5-HT_{2C} receptor antagonists in the improvement of bladder control should be approached with great caution.

Studies carried out on guinea pigs (McMurray & Miner, 2005) demonstrated the involvement of 5-HT_{2C} receptors in the control of bladder and urethral function. This study found that the selective 5-HT_{2C} receptor agonists Ro 60-0175 and YM348 significantly increased external urethral sphincter activity as well as inhibiting the micturition reflex. The selective 5-HT_{2C} receptor antagonist SB 242084 reversed the agonist induced activity on the urethral striated muscle and bladder function. The 5-HT_{2A} receptor antagonist MDL 100907 and 5-HT_{2B} receptor antagonist SB 204741 failed to affect the agonist induced responses on bladder and urethral function, thus drawing the conclusion that 5-HT_{2C} receptors are involved in mediating both bladder and urethral activity of these agonists in the guinea pig.

1.7.3 5-HT_3 receptors

The 5-HT_3 receptor is the only member of the 5-HT superfamily characterised as a ligand-gated ion channel (Derkach *et al.*, 1989). It was initially known as the 'M' receptor because it interfered with responses to morphine on induced gut contractions. Ligand-binding and functional studies have shown that the 5-HT_3 receptor is widely distributed in central and peripheral nervous tissue and the receptor has been reported to reduce alcohol consumption in experimental animals, whilst the antagonists of this receptor have been reported to treat anxiety disorders (see Bloom & Morales, 1998). The 5-HT_3 receptors have also been implicated in several gastrointestinal functions including peristalsis, intestinal secretion, sensation, nausea and vomiting (Andrews *et al.*, 1990; Gershon *et al.*, 1990).

1.7.3.1 Receptor distribution

5-HT_3 receptors were originally cloned from a neuroblastoma cell line (Maricq *et al.*, 1991). cDNA encoding a single 5-HT_3 receptor subunit (5-HT_{3A}) was initially identified and cloned from several mammalian species including mouse, rat, guinea pig and ferret (Mochizuki *et al.*, 2000; Lankiewicz *et al.*, 1998; Isenberg *et al.*, 1993). Another subunit, 5-HT_{3B} was also identified in both rat and human tissue (Davies *et al.*, 1999; Dubin *et al.*, 1999; Hanna *et al.*, 2000).

Several radioligand studies have mapped out the distribution of 5-HT_3 receptors in the CNS with the highest levels of the receptor binding sites present in the dorsal vagal complex in the brainstem (see Pratt *et al.*, 1990) consisting of the nucleus tractus solitarius, area postrema and dorsal motor nucleus of the vagus nerve. However, relative to the dorsal vagal nerve, 5-HT_3 receptor distribution is low in the forebrain.

High levels of the receptor are expressed in the hippocampus, amygdala and superficial layers of the cerebral cortex. Species variation has been found to exist with regards to the relative distribution of 5-HT_3 receptor distribution in the forebrain, where for example in humans, high levels of 5-HT_3 receptor recognition sites have been located within the caudate nucleus and putamen (Abi-Dargham *et al.*, 1993; Bufton *et al.*, 1993; Parker *et al.*, 1996), with relatively low levels detected within the cortical regions (Barnes *et al.*, 1989; Waeber *et al.*, 1988; Abi-Dargham *et al.*, 1993; Parker *et al.*, 1996). However, this pattern is reversed in rodents. In most species (e.g. mouse, rat, man; Parker *et al.*, 1996), relative to other forebrain regions, high levels of 5-HT_3 receptors have been found to be expressed in the hippocampus.
In peripheral tissues, 5-HT_3 receptors are primarily expressed in the gut wall. Glatzle *et al* (2002) demonstrated 5-HT_3 receptor expression by functionally distinct classes of neurones innervating the gastrointestinal tract. 5-HT_3 receptors were also observed on the cell surface as well as in the cytoplasm of the neurones. Furthermore, 5-HT_3 receptors were found in dense networks of fibers in the myenteric plexus, muscle and mucosa and also in the cell bodies and processes of interstitial cells of Cajal as well as in enteroendocrine cells. Few 5-HT_3 receptors were found to be expressed in enterochromaffin cells.

5-HT_3 receptors exist both as postsynaptic and presynaptic receptors. Postsynaptic receptors have been shown to be present on GABAergic interneurones and are thought to mediate fast synaptic transmission in the central nervous system (Sugita *et al.*, 1992; Lambert *et al.*, 1995). Furthermore, it has been suggested that presynaptic 5-HT_3 receptors modulate the release of several neurotransmitters in the central nervous system.

1.7.3.2 Receptor pharmacology

A large number of ligands that interact selectively with the 5-HT_3 receptor have been identified, thus enabling pharmacological definition of 5-HT_3 receptor evoked responses. The most commonly used 5-HT_3 receptor agonists include m-CPBG, phenylbiguanide and 2-methyl-5HT. However, highly selective 5-HT_3 receptor agonists have not been identified. Conversely, numerous highly potent 5-HT_3 receptor antagonists have been developed and these include granisetron, ondansetron, tropisetron (ICS 205930), zatosetron and MDL 72222. All these 5-HT_3 receptor antagonists have non-selective effects occurring at concentrations in excess of those required to antagonize 5-HT_3 receptors by 100-fold or more. These non-selective effects of 5-HT_3 receptor antagonists therefore include agonism of 5-HT_4 receptors and local anaesthetic-like block of ligand- and voltage-gated ion channels (Scholtysik *et al.*, 1988; Barann *et al.*, 1993; Ye *et al.*, 1997). An interesting phenomenon observed for the 5-HT_3 receptor is that inter-species pharmacological differences exist for the receptor. This is demonstrated by the fact that the antagonist MDL 72222 displays considerably lower affinity for the guinea pig variant of the 5-HT_3 receptor (Kilpatrick & Tyers, 1992; Lankiewicz *et al.*, 1998). Similarly, the partial 5-HT_3 receptor agonist m-CPBG differs approximately 300-fold between the rat and rabbit 5-HT_3 receptors (Kilpatrick *et al.*, 1990). Another pharmacologically distinct characteristic of the 5-HT_3 receptor is the existence of allosteric modulation of the receptor complex. This is demonstrated in studies where alcohol increases the potency with which agonists activate the 5-HT_3 receptor complex (Lovinger & Zhou, 1993; Downie *et al.*, 1995; Parker *et al.*, 1996). Furthermore, some anaesthetic agents have also been found to modify the function of the 5-HT_3 receptor (see Parker *et al.*, 1996).

1.7.3.3 Signal transduction

5-HT_3 receptors are non-selective cation channels that mediate transient inward currents and membrane depolarization under physiological conditions (Lambert *et al.*, 1995; see Barnes & Sharp, 1999). In the CNS, differences have been found to exist between the functional properties of the presynaptic and postsynaptic 5-HT_3 receptors. For example, presynaptic 5-HT_3 receptors on striatal synaptosomes are Ca^{2+}-permeable ion channels, and the influx of Ca^{2+} through these channels is responsible for the elevation of intracellular Ca^{2+} induced by stimulation of 5-HT (Rondé & Nichols; 1998). Conversely, postsynaptic 5-HT_3 receptors on hippocampal interneurones are blocked by Ca^{2+} at negative membrane potentials (McMahon & Kauer, 1997). Peripherally, the 5-HT_3 receptor has been shown to elicit a rapid depolarization produced by increased membrane permeability to monovalent cations in the gut (Peters & Lambert, 1989). In many enteric neurons, the 5-HT_3 receptor also mediates a rapidly developing and desensitizing depolarization (Galligan, 1996).

1.7.3.4 Physiological functions

The 5-HT_3 receptor is the only monoamine receptor to be associated with fast synaptic transmission in the brain (Sugita *et al.*, 1992). The presence of functional 5-HT_3 receptors in the brain was obtained from behavioural effects of the 5-HT_3 receptor ligands with much optimism generated towards the search of novel psychotropic agents. 5-HT_3 receptor antagonists have therefore been forwarded as potential therapeutic agents for disorders such as; anxiety, cognitive dysfunction and psychosis (see Bentley & Barnes, 1998). 5-HT_3 receptors have also been found to play an important role in the processing of cardiovascular afferent input at the level of the nucleus tractus solitarius (Jeggo *et al.*, 2005).

In peripheral tissues, 5-HT_3 receptors mediate a number of neural reflexes involved in the physiologic regulation of gastrointestinal function, including intestinal feedback, inhibition of gastric emptying (Raybould & Zittel, 1995) and gastrocolonic reflex (Foxx-Orenstein *et al.*, 1996). 5-HT_3 receptors have also been implicated in the defence of the gut for example in response to intestinal anaphylaxis (Cooke *et al.*, 1997; Mourad *et al.*, 1995).

1.7.3.5 5-HT_3 receptors – excitatory or inhibitory?

It has been documented that the 5-HT_3 receptor is associated with fast synaptic transmission in the brain (Sugita *et al.*, 1992), with fast synaptic depolarisations observed. Similarly, in the gut, the 5-HT_3 receptors receptor elicits a rapid depolarization produced by increased membrane permeability to monovalent cations (Peters & Lambert, 1989). This implies that 5-HT_3 receptors are excitatory as they mediate neuronal excitation characterized by depolarization thus increasing neuronal excitability and neurotransmitter release both centrally and peripherally.

1.7.3.6 5-HT_3 receptors and micturition

Pharmacological evidence implicating a role of the 5-HT_3 receptor in micturition was provided by a study carried out by Espey and Downie (1995) where intrathecal administration of the 5-HT_3 receptor antagonist zatosetron decreased volume threshold in spinally intact conscious cats. Additionally, the so called 5-HT_3 receptor agonist 2-methyl increased volume threshold in cats that had undergone complete spinal transection. From these results, the authors concluded that spinal 5-HT_3 receptors were physiologically involved in micturition by mediating inhibitory effects.

A subsequent study by Espey *et al* (1998) recorded ascending activity (bladder afferent activation) evoked by pelvic nerve stimulation in the thoracic spinal cord of anaesthetized cats. The group observed that following intrathecal administration of 5-HT_3 receptor antagonists zatosetron, tropisetron (ICS 205930) and MDL 72222, pelvic evoked neuronal activity was increased in spinally intact cats. These results suggested that through 5-HT_3 receptor activation, endogenous serotonin tonically inhibited ascending activity in spinally intact cats. This was further confirmed by the decrease in volume threshold observed following intrathecal administration of zatosetron (Espey & Downie, 1995). These data therefore support the theory that by increasing ascending activity through blockade of spinal 5-HT_3 receptors the levels of spinal 5-HT are decreased. Preganglionic parasympathetic neurones are thereafter activated and this result in the excitatory effect observed on micturition. Conversely, activation of 5-HT_3 receptors by 2-methyl-5-HT depressed ascending activity thus implying that spinal 5-HT is increased and activation of the endogenous spinobulbospinal negative feedback mechanism results in inhibition of the micturition reflex. Interestingly, intrathecal administration of 2-methyl-5-HT increased the pelvic-pudendal reflex whereas zatosetron inhibited this reflex (Espey *et al*, 1998) thus suggesting the involvement of 5-HT_3 receptors in the control of the EUS in the conscious cat. I.v. administration of zatosetron and Y 25130 had no effect on isovolumetric bladder contractions in anaesthetized rats (Testa *et al.*, 2001). Similarly, i.c.v administration of 2-methyl-5-HT also had no effect on the cystometry parameters in conscious rats (Ishizuka *et al.*, 2002).

1.7.4 5-HT_4

Activation of the 5-HT_4 receptor mediates widespread effects in both central and peripheral nervous systems, through the use of selective agonists and antagonists. Centrally, the receptor has been implicated in the regulation of learning and memory, cognition and anxiety (Eglen *et al.*, 1995). Activation of the 5-HT_4 receptor peripherally has been shown to play a modulatory role in several tissues including heart, gastrointestinal tract, adrenal gland and the urinary bladder (Hegde & Eglen, 1996).

1.7.4.1 Receptor distribution

Development of specific radioligands has enabled mapping of the central distribution of the 5-HT_4 receptor. Relatively high levels of 5-HT_4 receptors have been identified in the nigrostriatal and mesolimbic systems of the brain of various species including rat, guinea pig, pig, cow, monkey and humans (Grossman *et al.*, 1993; Waeber *et al.*, 1993; Doménech *et al.*, 1994; Jakeman *et al.*, 1994; Schiavi *et al.*, 1994; Mengod *et al.*, 1996). In the limbic system, the 5-HT_4 receptors are localized in the olfactory tubercle, frontal cortex, fundus striatus, ventral pallidum, septal region, hippocampus and amygdala (see Eglen *et al.*, 1995). It should be noted that species variation exists with regards to the distribution of the 5-HT_4 receptor, where for example more abundant receptors are found in the interpendicular nucleus, habenula, lateral substantia nigra, medial hypothalamus and locus coeruleus of the rat compared to the guinea pig (Waeber *et al.*, 1993; Jakeman *et al.*, 1994). In the periphery, 5-HT_4 receptors are found to be expressed in rat ileum, colon, bladder and in the atrium (Gerard *et al.*, 1995). RT-PCR studies have revealed 5-HT_4 receptor expression on

human endothelial cells and in many blood vessels studied from the pig (Ullmer *et al.*, 1995).

In situ hybridization histochemistry studies revealed that 5-HT_4 receptors were located both somatodendritically and axonally in both the rat and guinea pig (Vilaro *et al.*, 2005).

1.7.4.2 Receptor pharmacology

The lack of selectivity towards the 5-HT_4 receptor restricts *in vivo* studies of the receptor. Agonists such as BIMU1, BIMU8, RS 66331 and RS 56532 have all been used as 5-HT_4 receptor agonists but unfortunately these compounds also possess equal or higher affinity for the 5-HT_3 receptor (Waeber *et al.*, 1993; Schiavi *et al.*, 1994; Domenech *et al.*, 1994). This is also the case for compounds such as zacopride, renzapride and cisapride (Domenech *et al.*, 1994). However, 5-HT_4 receptor agonists SC-53116 and SC-49518 possess lower affinity for the 5-HT_3 receptor thus making these agonists more selective for the 5-HT_4 receptor. Another highly potent, selective, partial agonist at 5-HT_4 receptors in guinea pig ileum and rat oesophagus is ML10302. Additionally, RS 67333 and RS 67506 which exhibit similar high affinities are also selective, but are also partial agonists for the 5-HT_4 receptor.

Previously, antagonists such as SDZ205557 and DAU6285 were used as 5-HT_4 receptor antagonists but these compounds exhibit similar affinities at the 5-HT_3 receptor. Numerous compounds have now been developed that are highly selective at the 5-HT_4 receptor. GR 113808 has been extensively used to characterize the 5-HT_4 receptor *in vitro* (Bockaert *et al.*, 1992). However, this compound is rapidly degraded

in vivo and therefore its analogue GR 125487 is the compound of choice when investigating 5-HT_4 receptors *in vivo* (Eglen *et al.*, 1994). Other selective 5-HT_4 receptor antagonists that have been found to be suitable for *in vivo* studies include RS 39604 (Hedge *et al.*, 1994), RS 100235, RS 67532 (Bockaert *et al.*, 1992) and SB 207266 (Wardle *et al.*, 1996).

1.7.4.3 Signal transduction

Activation of 5-HT_4 receptors leads to the activation of adenylate cyclase, thereby increasing intracellular cAMP levels (Ford & Clarke, 1993). It has been revealed that 5-HT_4 receptor-mediated increase in cAMP levels may lead to phosphorylation of a range of target proteins e.g. cAMP-dependent protein kinase A (Fagni *et al.*, 1992). This phosphorylation of the voltage-gated K^+ channels leads to their closure. Moreover, in dorsal root ganglion cells, 5-HT_4 receptor activation is associated with an increase in tetrodotoxin-insensitive Na^+ current (Cardenas *et al.*, 1997). It should be noted that there are differences in the coupling of 5-HT_4 receptors between tissues. Therefore, in mouse colliculi neurones, compounds may act as full agonists following measurements of intracellular cAMP (Dumuis *et al.*, 1988) whereas in the hippocampus, the same compounds act as partial agonists. Additionally, at 5-HT_4 receptors coupled to inward K^+ currents in the rat hippocampus, these compounds act as antagonists (Chaput *et al.*, 1990).

1.7.4.4 Physiological functions

5-HT_4 receptors in the central nervous system have been implicated in the regulation of learning and memory, cognition and anxiety (Eglen *et al.*, 1995). Receptor distribution in the brain has enabled the effects of 5-HT_4 receptor agonists and

antagonist assessment in cognitive impairment. Peripherally, activation of the 5-HT_4 receptor plays a modulatory role in several tissues including heart, gastrointestinal tract, adrenal gland and urinary bladder (Hegde & Eglen, 1996). In the gastrointestinal tract, effects such as motility, peristaltic reflex and secretion have been studied in the gut of different species using various 5-HT_4 receptor agonists and antagonists (see Hedge & Eglen, 1996). In the cardiovascular system, activation of 5-HT_4 receptors by 5-HT in humans and pigs induces tachycardia and positive inotropy (see Kaumann, 1994). 5-HT_4 receptors have also been implicated in the pathogenesis of arrhythmias leading to atrial fibrillation and stroke (see Kaumann, 1994). The only known endocrine effect of the 5-HT_4 receptor was first demonstrated in the frog adrenal gland and later in the human adrenal cortex (Idres *et al.*, 1991; Contesse *et al.*, 1994; Lefebvre *et al.*, 1993) where coticosterone and aldosterone release was shown to be mediated by the 5-HT_4 receptor.

1.7.4.5 5-HT_4 receptors – excitatory or inhibitory?

Electrophysiological studies have demonstrated 5-HT_4 receptor activation in neurones increases excitability (Chaput *et al.*, 1990; Andrade & Chaput, 1991; Roychowdhury *et al.*, 1994). In rat hippocampus and mouse colliculi neurones, intracellular accumulation of cAMP modulates the activity of protein kinase A which leads to the closure of K^+ channels. The closure of K^+ channels results in a reduction in repolarization thus increasing neuronal excitability and ultimately, increasing neurotransmitter release (Ansanay *et al.*, 1995).

1.7.4.6 5-HT_4 receptors and micturition

Tonini *et al* (1994) demonstrated a facilitatory role for 5-HT_4 receptors in cholinergic mediated contractions of the human bladder. The group found that in postganglionic cholinergic nerves, 5-HT caused concentration-dependant increases in the amplitude of contractions in human isolated detrusor strips in methylsergide and ondansetron pretreated tissues. These concentration-dependant increases were antagonized by the selective 5-HT_4 receptor antagonist GR 113808 in a competitive manner and therefore the authors were able to conclude that cholinergic transmission in human isolated detrusor muscle was facilitated by 5-HT_4 receptors. Another study providing evidence for the involvement of 5-HT_4 receptors in bladder function was demonstrated by Waikar *et al* (1994). The group found that 5-HT inhibited electrically evoked contractions of isolated urinary bladder strips from Rhesus and Cynomolgus monkeys. The 5-HT_4 receptor antagonists, GR 113808 and DAU 6285 produced parallel displacement effects of the concentration-effect curves of 5-HT, thus suggesting that this inhibitory effect was mediated via activation of 5-HT_4 receptors. In anaesthetized rats, i.v. administration of the 5-HT_4 receptor antagonist RS 39604 did not affect regular isovolumetric bladder contractions (Testa *et al.*, 2001). Conversely, the 5-HT_4 receptor agonist RS 67506 administered i.c.v. to conscious rats was observed to decrease bladder capacity and micturition volume (Ishizuka *et al.*, 2002) thus suggesting that in normal conscious rats, at the supraspinal level, 5-HT_4 receptors can enhance the micturition reflex induced by bladder filling. However, further studies need to be carried out in order to deduce the mechanism and site of action of these receptors in micturition. Therapeutic applications of the 5-HT_4 receptor in the human urinary bladder have originated from the use of 5-HT_4 receptor agonists such as cisapride, whose side effect is known to include increased micturition

frequency/urinary incontinence (Boyd & Rohan, 1994). This effect is thought to be due to the strategic placement of 5-HT_4 receptors on cholinergic nerve terminals. Similarly, metoclopramide which possesses selectivity for the 5-HT_4 receptor has been reported to diminish residual urine volume in a diabetic patient with a neurogenic bladder (Nestler *et al.*, 1983) thus suggesting that 5-HT_4 receptor agonists may be of value in the treatment of bladder disorders associated with detrusor hypomotility such as overflow incontinence.

1.7.5 5-ht_{5A} receptors

The 5-ht_5 receptor family consists of 5-ht_{5A} and 5-ht_{5B} receptors which share approximately 68% mutual amino acid identity (Plassat *et al.*, 1992; Erlander *et al.*, 1993). These two 5-ht_5 receptors have been identified in both mouse (Plassat *et al.*, 1992; Matthes *et al.*, 1993) and rat (Erlander *et al.*, 1993). However in humans, only the 5-ht_{5A} receptor gene encodes a functional protein whereas the 5-ht_{5B} encodes a non-functional protein due to its interruption by stop codons. The physiological function of the 5-ht_{5A} receptor is not well understood due to a lack of specific ligands, but it has been implicated in the control of circadian rhythm, mood disorders and schizophrenia and other cognitive disorders.

1.7.5.1 Receptor distribution

mRNA localisation studies in mouse (Plassat *et al.*, 1992; Matthes et al., 1993), rat (Erlander *et al.*, 1993) and human (Rees *et al.*, 1994; Pasqualetti *et al.*, 1998) brain have revealed evidence for 5-ht_{5A} receptor mRNA in cerebral cortex, hippocampus, thalamus, hypothalamus, habenula, cerebellum and spinal cord. Additionally, immunohistochemical studies in the rat have also confirmed the presence of 5-ht_{5A} receptor in raphe nuclei (dorsal and median raphe), amygdala and hypothalamus (Oliver *et al.*, 2000). In several of these regions, the receptor is expressed on neurones which include cortical and hippocampal pyramidal neurones, purkinje neurones, molecular layer interneurones and Golgi cells of the cerebellum (Geurts *et al.*, 2002). Immunolocalisation studies have also confirmed expression of 5-ht_{5A} receptor in the suprachiasmatic nucleus (Duncan *et al.*, 2000) in hamsters.

In the periphery, 5-ht_{5A} receptor mRNA expression was observed as very low to absent. This was observed following the use of RT-PCR technique (Rees *et al.*, 1994)

where 5-ht_{5A} receptor mRNA was found to be absent in human heart, kidney, liver, small intestine, spleen and uterus. However, 5-ht_{5A} receptor mRNA was observed to be present in rat superior cervical ganglion, petrosal ganglion and carotid body (Wang *et al.*, 2000).

The 5-ht_{5A} receptor has been shown to be exclusively localized on postsynaptic cells in all the regions expressing the receptor (Doly *et al.*, 2004).

1.7.5.2 Receptor pharmacology

A complication with the 5-ht_{5A} receptor is that no selective ligands have been identified for the receptor. An additional complicating factor is that pharmacological similarities have been observed for the 5-ht_{5A} receptor when compared to either 5-HT_7 or 5-HT_{1A} receptor. For example, like the 5-HT_{1A} receptor, the 5-HT_{5A} receptor displays a high affinity for 5-carboxamidotryptamine (5-CT) and is potently antagonized by non-selective 5-HT receptor antagonist methiothepin (see Thomas, 2006). The only way of distinguishing between the two receptors however, is through the use of the 5-HT_{1A} receptor antagonist WAY 100635 which exhibits low affinity for the 5-ht_{5A} receptor. 5-ht_{5A} and 5-HT_7 receptors are known to display a pharmacological overlap where both receptors share the same rank order of agonist potency; 5-CT>5-HT>8-OH-DPAT. They are also both antagonized by 5-HT receptor antagonists methiothepin and ritanserin (Thomas *et al.*, 1999, Thomas *et al.*, 2004). Furthermore, antagonist studies using the selective 5-HT_7 receptor antagonist SB-269970 have demonstrated moderate high affinity of the compound for the 5-ht_{5A} receptor (pK_i 7.2). Similarly, the potent 5-HT_7 receptor antagonist SB-656104 and close analogue of SB-269970 also displays affinity for the 5-ht_{5A} receptor (pK_i 6.7),

although this is lower (Forbes *et al.*, 2003; Thomas *et al.*, 2003; Thomas & Hagan, 2004). Recently, the first selective 5-ht_{5A} receptor antagonist SB-699551 has been developed (Corbett *et al.*, 2005). This compound is at least 30-fold selective for the 5-ht_{5A} receptor versus the other 5-HT receptor subtypes. What is interesting about this compound however is that the pharmacological profile is species dependant where it exhibits a high affinity for the human and guinea pig 5-ht_{5A} receptor, but low affinity for the rat and mouse receptors (see Thomas, 2006).

1.7.5.3 Signal transduction

Radioligand binding studies with the recombinant 5-ht_{5A} receptors have provided evidence to indicate that the receptors couple to a G-protein. The 5-ht_{5A} receptor appears to couple preferentially to $G_{i\,o}$ proteins as agonist-induced activation of the receptor inhibits adenylyl cyclase activity in various cell systems (Francken *et al.*, 1998, 2001; Thomas *et al.*, 2004). Additionally, the receptor has also been reported to couple inwardly to rectifying K^+ channels ($GIRK_1$) when expressed in oocytes (Grailhe *et al.*, 2001). When expressed in C6 glioma cells, the 5-ht_{5A} receptor has been shown to enhance intracellular Ca^{2+} signaling (Noda *et al.*, 2003). It should be noted however that the transduction mechanisms described above have only been observed for the recombinant 5-ht_{5A} receptor, whereas mechanisms by which the receptor couples to signaling pathways in native tissues remains to be established.

1.7.5.4 Physiological functions

Tissue localisation of the 5-ht_{5A} receptor in the dorsal and median raphe as well as the suprachiasmatic nucleus suggests a potential role for the 5-ht_{5A} receptor in the control of circadian timing. The 5-ht_{5A} receptor has also been implicated as a potential drug

target to treat mood disorders including anxiety and depression as well schizophrenia and other cognitive disorders. It has also been postulated that the 5-ht_{5A} receptor may play a role in central motor control, nociception and autonomic function (Doly *et al.*, 2004).

1.7.5.5 5-ht_{5A} receptors – excitatory or inhibitory?

As mentioned above, it has been reported that the 5-ht_{5A} receptor couples inwardly to rectifying K^+ channels ($GIRK_1$) when expressed in oocytes (Grailhe *et al.*, 2001). It was previously reported that activation of GIRK channels causes membrane hyperpolarisation and therefore the channels play an important role in the inhibitory regulation of neuronal excitability (North & Uchimura, 1989). One can therefore speculate that following activation of $GIRK_1$ channels by the 5-ht_{5A} receptor, inhibitory effects are observed on neuronal excitability.

1.7.5.6 5-ht_{5A} receptors and micturition

Spinal motorneurones of the Onuf's nucleus innervating pelvic striated muscles are involved in direct control of pelvic function, including micturition via the external urethral sphincter. Species variation exists with regards to the location of the Onuf's nucleus. In rats, studies have demonstrated that the Onuf's nucleus is located in the L5-L6 spinal segments and consists of two separate nuclei, namely the dorsomedial nucleus and the dorsolateral nucleus (McKenna & Nadelhaft, 1986). Xu and colleagues (2007) observed intense 5-ht_{5A} receptor immunoreactivity in the dorsolateral nucleus in both male and female rats. Both light and electron microscopy studies revealed labelling of the dorsolateral nucleus following injections of horseradish peroxidase in the external urethral sphincter. Similarly, injections of

pseudorabies virus into the external urethral sphincter also revealed labelling of the dorsolateral nucleus. This labelling study clearly reveals that 5-ht_{5A} receptors are associated with external urethral sphincter motorneurones in male and female rats on the dorsolateral nucleus of the Onuf's nucleus. The external urethral sphincter has been found to play a major role in continence by maintaining closure of the urethral sphincter. From the results above, it would appear that 5-ht_{5A} receptors may be selectively involved in the control of continence in both male and female rats.

1.7.6 5-HT_6 receptors

The existence of 5-HT_6 receptors was revealed primarily from the use of molecular cloning techniques. Cloning of the 5-HT_6 receptor has therefore been demonstrated in the rat, human and mouse tissue (Monsma *et al.*, 1993; Ruat *et al.*, 1993; Kohen *et al.*, 1996, 2001).

1.7.6.1 Receptor distribution

Northern blots have shown that 5-HT_6 receptor mRNA appears to be exclusively located in the brain, with very little evidence for its presence in peripheral tissues. In situ hybridization, northern blot analysis, and RT-PCR have revealed the presence of the 5-HT_6 receptor in the olfactory tubercle, nucleus accumbens, striatum, hippocampus and cerebral cortex in the rat brain (Monsma *et al.*, 1993; Ruat *et al.*, 1993; Ward *et al.*, 1995, Gerard *et al.*, 1997; Grimaldi *et al.*, 1998). Lower levels of the receptor have also been detected in the granular layer of the cerebellum, several diencephalic nuclei and amygdala (Ward *et al.*, 1995). Additionally, 5-HT_6 receptor immunoreactivity associated with dendritic processes in striatum and hippocampus of the adult rat has been demonstrated using immunocytochemistry (Gerard *et al.*, 1997). In the human brain however, highest expression levels of 5-HT_6 receptor mRNA were found in the caudate nucleus (Kohen *et al.*, 1996). Interestingly, East *et al* (2002) revealed distribution patterns similar to rats, in postmortem samples from schizophrenic and normal patients.

Information regarding peripheral distribution of 5-HT_6 receptor distribution in peripheral tissues is very limited. Northern blot analysis of stomach rat tissue detected low expression levels of 5-HT_6 receptors (Ruat *et al.*, 1993). In the guinea

pig, northern blot analysis revealed a very faint signal of 5-HT_6 receptor expression in the adrenal glands (Ruat *et al.*, 1993). RT-PCR has also revealed 5-HT_6 receptor mRNA expression in *ex vivo* isolated spleen, thymus and peripheral blood lymphocytes in rat tissue which would suggest the possibility of the involvement of 5-HT_6 receptors on immune functions (Stefulj *et al.*, 2000).

Immunocytochemistry studies revealed 5-HT_6 receptor-like immunoreactivity was associated with dendritic processes making synapses with unlabelled axonal synapses in both hippocampus and striatum (Gerard *et al.*, 1997). This would therefore suggest that 5-HT_6 receptors in these regions are predominantly postsynaptic.

1.7.6.2 Receptor pharmacology

The 5-HT_6 receptor possesses unique pharmacological profiles as it has high affinities for non-selective agents such as tryptamines, methiothepin, lisuride, LSD and clozapine. Similarly, the 5-HT_6 receptor also exhibits high affinity for tricyclic antidepressants as well as a large number of typical and atypical antipsychotic agents (Roth *et al.*, 1994). For many years, there was a lack of selective agonists and antagonists at the receptor, therefore the non-selective compounds mentioned above e.g. clozapine (Glatt *et al.*, 1995) were used to label the 5-HT_6 receptor. This meant that characterization of 5-HT_6 receptor mRNA in native tissue was difficult as the compounds used had selectivity for dopamine receptors, monoamine oxidases as well as other 5-HT receptors. To date, three selective 5-HT_6 agonists have been developed and these include EMDT, LY586713, and WAY-466 (Glennon *et al.*, 2000; Bernotas *et al.*, 2004). Several antagonists have also been developed in recent years for the 5-HT_6 receptor with the first reported antagonists being Ro 04-6790 and Ro 63-0563

(Sleight *et al.*, 1998). Both these antagonists were reported to be potent at both rat and human recombinant 5-HT_6 receptors. Additionally, another 5-HT_6 receptor antagonist SB 271046 (Hirst *et al.*, 2003) was developed, and it is thought that this compound may be a significant new tool for the study of 5-HT_6 receptor function, due to good selectivity for the receptor as well as its low blood clearance, good half life and oral bioavailability. The antagonist radioligand [^{125}I]-SB-258585 (Hirst *et al.*, 2000) was also identified and utilized in characterizing the 5-HT_6 receptor.

1.7.6.3 Signal transduction

The 5-HT_6 receptor was identified as the first mammalian serotonin receptor to be cloned that was coupled to activation of adenylyl cyclase (Monsma *et al.*, 1993; Ruat *et al.*, 1993; Kohen *et al.*, 1996; Boess *et al.*, 1997). The recombinant 5-HT_6 receptor expressed in various artificial expression systems couples to the G stimulatory (G_s protein). Subsequent studies using striatal tissues and mouse neuroblastoma have also revealed that native 5-HT_6 receptors are positively coupled to adenylyl cyclase (Schoeffter & Waeber, 1994; Sebben *et al.*, 1994).

1.7.6.4 Physiological functions

Due to the unique pharmacological profile of the 5-HT_6 receptor, primary interest in the receptor has been based on the pathology and treatment of depression and/or affective disorders such as schizophrenia. Additionally, high levels of the 5-HT_6 receptor have been detected in the hippocampus, and a role of the 5-HT_6 receptor in memory, especially hippocampal-dependent aspects of learning and memory has been postulated (Perez-Garcia & Meneses, 2005). The 5-HT_6 receptor has been shown to influence acetylcholine release in the frontal cortex (Riemer *et al.*, 2003) thus

suggesting a role for the receptor in cognition deficits such as Alzheimer Disease (AD). Regarding a role of the 5-HT_6 receptor in anxiety or depression, very few studies in the literature have explored the role of 5-HT_6 receptor activity in these two conditions. Yoshioka *et al* (1998) showed that increased 5-HT in the prefrontal cortex from conditioned fear is significantly inhibited by treatment with antisense oligonucleotides, specific to 5-HT_6 mRNA infused i.c.v. In a later study, Hamon *et al* (1999) demonstrated that infusion of antisense oligonucleotides specifically down-regulated 5-HT_6 receptor expression in the nucleus accumbens with increased anxiety observed in the elevated plus maze and the social interaction test. As 5-HT_6 receptors are present in various limbic areas, it was suggested (Monsma *et al.*, 1993; Roth *et al.*, 1994) that 5-HT_6 receptors play an important role in mediating the actions of antipsychotic drugs. It is also known that antipsychotic compounds such as clozapine and olanzapine exhibit a strong affinity for 5-HT_6 receptors. It has been demonstrated that clozapine significantly reduced 5-HT_6 mRNA in the hippocampus (Frederick & Meador-Woodruff, 1999) and down-regulated 5-HT_6 receptors expressed in cultured cells (Zhukovskaya & Neumaier, 2000) thus providing evidence for a physiological role for 5-HT_6 receptors in schizophrenia.

1.7.6.5 5-HT_6 receptors – excitatory or inhibitory?

Due to a lack of receptor-like immunoreactivity in the soma of excitatory pyramidal and granule cell neurones in the hippocampus Gerard and co-workers (1997) postulated that 5-HT_6 receptors were probably located on the dendrites of these neurones. The presence of 5-HT_6 receptors in dendrites of the above mentioned cell neurones suggest that 5-HT_6 receptors may possibly be excitatory on the hippocampus. Conversely, Ward and Dorsa (1996) demonstrated 5-HT_6 receptor

expression on dendritic processes of GABAergic neurones terminating in the substantia nigra and globus pallidus. The above neurones are thought to exert differential modulation (inhibition and dishinhibition, respectively) of basal ganglia in the substantia nigra (see Barnes & Sharp, 1999) thus suggesting the receptors mediate inhibitory effects.

1.7.6.6 5-HT_6 receptors and micturition

To my knowledge, there is only one study in the literature that has looked at the effects of a selective 5-HT_6 receptor ligand and its effect on micturition. This study was carried out by Testa and colleagues (2001) where they found that i.v. administration of the 5-HT_6 receptor antagonist Ro 04-6790 was ineffective on regular isovolumetric bladder contractions. From these results, the group concluded that 5-HT_6 receptors are of little importance for control of micturition.

1.7.7 5-HT$_7$ receptors

The 5-HT$_7$ receptor is the most recently identified member of the family of G-protein-coupled 5-HT receptors. The use of non selective ligands has provided evidence for the involvement of the 5-HT$_7$ receptor in regulation of the limbic system, sensory processing and circadian rhythms as well as a possible involvement in migraines and afferent sympathetic pathways (see Vanhoenacker *et al.*, 2000).

1.7.7.1 Receptor distribution

In the rat and guinea pig, the 5-HT$_7$ receptor subtype is unique in that both the mRNA and receptor binding sites display a similar distribution site, thus suggesting that the receptor is expressed very closely to the site of synthesis (Gustafson *et al.*, 1996; Stowe & Barnes, 1998). 5-HT$_7$ receptor expression has been found to be elevated within regions of the thalamus, hypothalamus, cortical regions and hippocampus (Gustafson *et al.*, 1996; Stowe & Barnes, 1998).

Alternative splicing in human and rat tissues has produced several 5-HT$_7$ receptor isoforms which include 5-HT$_{7(a)}$, 5-HT$_{7(b)}$, 5-HT$_{7(c)}$. A difference exists between the distribution patterns of the human 5-HT$_{7(b)}$ receptor isoforms when compared to the rat. In both species, 5-HT$_{7(a)}$ is predominantly found in the spleen, kidney, heart, thalamus, hindbrain, hippocampus, cortex, caudate, striatum and cerebellum (Monyer & Lambolez, 1995). With the 5-HT$_{7(b)}$ isoform, 31-45% of the human 5-HT$_7$ receptor mRNA is present in the caudate, hippocampus and spleen compared to 14-18% in the corresponding rat tissues. No differences have been noted with regards to the distribution of the 5-HT$_{7(c)}$ receptor isoform in either of the two species.

1.7.7.2 Receptor pharmacology

Non selective receptor agonists such as [^{3}H]5-HT, [^{3}H]5-CT and receptor antagonists such as [^{3}H] risperodine have been used to further characterise the 5-HT$_7$ receptor. However at present there are no commercially available selective 5-HT$_7$ receptor agonists. SB258719 is the first 'selective' 5-HT$_7$ receptor antagonist described and has been shown to be at least 100-fold more selective for the 5-HT$_7$ receptor compared to other 5-HT receptors. In addition, Meiji Seika Kaisha Ltd synthesised another 'selective' 5-HT$_7$ receptor antagonist DR4004 with a 47-fold selectivity over the 5-HT$_2$ receptor and others (Kikuchi *et al.*, 1999). The most selective 5-HT$_7$ receptor antagonist developed to date is SB-269970 which is known to possess a profile greater than 100-fold selectivity for other receptors (except 5-ht$_{5A}$ receptors which is 50-fold) (see Wesolowska, 2002). Defining the above compounds as selective 5-HT$_7$ receptor antagonists should be done with caution as not all 5-HT receptor subtypes have been used to determine the receptor binding profile, thus they are not 'truly' selective for the 5-HT$_7$ receptor. Interestingly, no major pharmacological differences have been identified between the different 5-HT$_7$ receptor isoforms.

1.7.7.3 Signal transduction

The 5-HT$_7$ receptor preferentially activates adenylate cyclase by supposedly coupling to $G_s\alpha$ (Adham *et al.*, 1998). Stimulation of the 5-HT$_7$ receptor with $G_s\alpha$ was found to mediate the G_s sensitive form of adenylate cyclase i.e. adenylate cyclase 5. Interestingly, coupling of the artificially expressed 5-HT$_{7(a)}$ receptor isoform with $G_s\alpha$ not only mediated the G_s sensitive form of adenylate cyclase , but also the G_s insensitive forms, adenylate cyclase 1 and adenylate cyclase 8 (Baker *et al.*, 1998). It

has been postulated that an increase in intracellular calcium may be responsible for the 5-$HT_{7(a)}$ receptor-mediated activation of adenylate cyclase 1 and adenylate cyclase 8. However, activation following an increase in phosphatidylinositol turnover or G_i activation has been ruled out as factors mediating the above events (Baker *et al.*, 1998).

1.7.7.4 Physiological functions

The 5-HT_7 receptor has been implicated in the regulation of circadian rhythms. This is based on studies done using 5-CT and 8-OH-DPAT where both agonists possess selectivity at the 5-HT_7 receptor (Lovenberg *et al.*, 1993). The expression of mRNA for 5-HT_7 receptors in the thalamic and limbic structures suggests that the receptors may be involved in affective behaviour. Interestingly, a conflict of interest exists with regards to the 5-HT_7 receptors and their involvement in schizophrenia. Clinical studies carried out on schizophrenic patients have shown a decrease in 5-HT_7 mRNA in the prefrontal cortex thus suggesting that 5-HT_7 receptors may be involved in schizophrenia (Vanhoenacker *et al.*, 2000). However, another study reported that no differences in 5-HT_7 mRNA were observed in the hippocampus of subjects with chronic schizophrenia (Erdmann *et al.*, 1996). A possible role for 5-HT_7 receptors in the treatment of epilepsy has also been deduced as shown in a study where non-selective 5-HT receptor antagonists preventing 5-HT-induced activation of adenylate cyclase by heterologously expressed 5-HT_7 receptors correlated with their ability to prevent seizures in mice (Bourson *et al.*, 1997)

1.7.7.5 5-HT_7 receptors – excitatory or inhibitory?

Not many studies have been carried out to establish whether 5-HT_7 receptors are excitatory or inhibitory on neuronal firing in the central nervous system. However, 5-HT_7 receptors are thought to mediate the inhibitory effect of 5-HT on purkinje neurones in the cerebellum. This conclusion is drawn from the fact that purkinje neuronal activity is inhibited following iontophoretic application of the agonists 8-OH-DPAT and ipsapirone (Darrow *et al.*, 1990), of which both possess affinity for the 5-HT_7 receptor. Conversely, a study by Roberts and colleagues (2004) suggested that 5-HT_7 receptor activation exerted a stimulatory rather than inhibitory influence on dorsal raphe 5-HT neurons. From these results, it is evident that the excitatory or inhibitory effects of the 5-HT_7 receptors is greatly dependant on their location.

1.7.7.6 5-HT_7 receptors and micturition

It is common knowledge that the receptor agonist and antagonists for the 5-$HT_{1A/2}$ receptors also possess affinity for the 5-HT_7 receptor. These include agonists such as 8-OH-DPAT and mCPP and antagonists such as WAY 100635, methylsergide (Krobert & Levy, 2002), methiothepin and mesulergine (Hagan *et al.*, 2000).

Mesulergine has been used to label 5-HT_7 receptors in the rat brain and is therefore considered to possess good selectivity for the 5-HT_7 receptor. Palea *et al* (2004) found that a correlation existed between the potency for mesulergine in the rat bladder (pA_2 = 7.27) when compared to the potency of functional 5-HT_7 receptors in the rat thalamus. However no correlation was observed when both 5-HT_{2A} and 5-HT_{2C} receptors were investigated in this study.

The development of 5-HT_7 receptor antagonists SB-269970 and SB-656104 which are from the same chemical series but are structurally distinct has enabled further investigation of the role of 5-HT_7 receptors in micturition. Read *et al* (2003) observed inhibition on the micturition reflex following i.c.v. administration of SB-269970 and SB-656104. Interestingly, i.t. (L6/S1 region of spinal cord) and i.v. administration of SB-269970 had no significant effects on any of the bladder variables measured. Conclusions drawn from these results were that these antagonists inhibited the micturition reflex by blocking central 5-HT_7 receptors. Most importantly, these results also confirmed that 5-HT_7 receptors located in the forebrain were involved in the control of bladder function, whereas the receptors in the sacral spinal cord were not. Interestingly, both compounds displayed no significant effects on the reflex-evoked urethral function thus suggesting that central 5-HT_7 receptors were not involved in mediating this effect. Surprisingly, central administration of SB-656104 significantly increased residual volume suggesting there was interference of the urethra. This however was not mirrored by the more selective 5-HT_7 receptor agonist SB-269970. Overall these findings provide evidence for the involvement of central 5-HT_7 receptors in the control of micturition.

1.8 Representation of the putative supraspinal 5-HT_{1A} autoreceptor pathway involving a 5-HT_{2C} receptor

WAY 100635 has been shown to be an antagonist at both the pre- and postsynaptic level (Fletcher *et al.*, 1996) and is thus considered to be a full 5-HT_{1A} receptor antagonist. Testa *et al* (1999) observed significant inhibition of rhythmic bladder contractions following i.v. administration of WAY 100635. Interestingly, this inhibitory effect of WAY 100635 was antagonized by pretreatment with the 5-HT_2 receptor antagonist mesulergine, whereas the 'partial agonist' NAN-190 was unaffected. Similar results were reported from interaction studies in rats where pretreatment of WAY 100635 with the 5-HT_{2A} receptor antagonist ketanserin was devoid of inhibitory effects. These results clearly suggest the involvement of 5-HT_2 receptors. A putative supraspinal 5-HT_{1A} receptor pathway involving the 5-HT_{2C} receptor was proposed as the mechanism responsible for blockade of the effects of WAY 100635 on the micturition reflex by mesulergine (see de Groat, 2002). Although 5-HT_{1A} receptors are universally located in the rat brain, cell clusters located in the raphe nuclei have been identified which contain abundant somatodendritic 5-HT_{1A} autoreceptors (see Barnes & Sharp, 1999). There are several studies in the literature providing evidence supporting the idea that WAY 100635 acts on supraspinal 5-HT_{1A} receptors unmasking an inhibitory pathway that is tonically suppressed by supraspinal serotonergic mechanisms. Therefore in micturition, WAY 100635 is thought to block 5-HT_{1A} inhibitory autoreceptors in the raphe neuron, which in turn enhances neuronal firing and increases the release of 5-HT in the spinal cord. An increase in spinal 5-HT activates the endogenous spinobulbospinal negative feedback mechanism, thus leading to inhibition of the micturition reflex. Blockade of

the effects of WAY 100635 by mesulergine is thought to occur as a result of spinal 5-HT activating excitatory 5-HT_{2C} receptors on segmental inhibitory interneurones which in turn suppress parasympathetic preganglionic neurons.

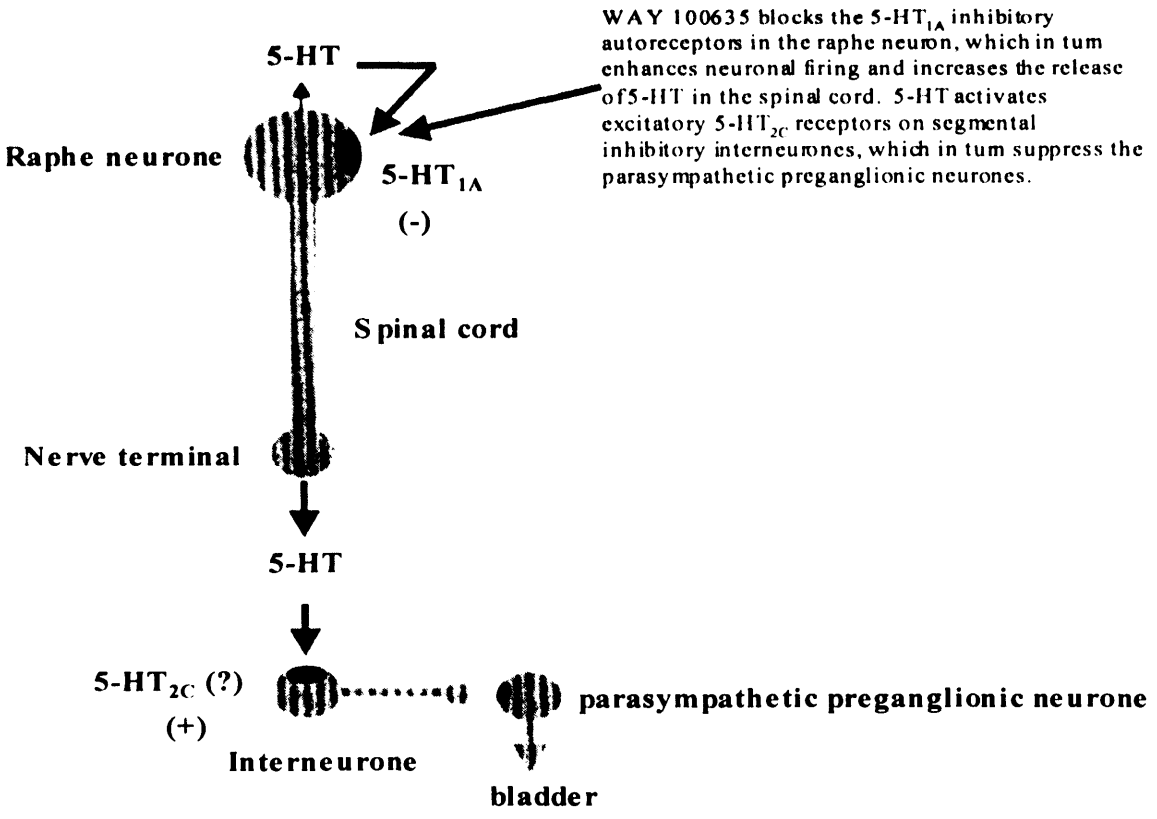

Figure 1.3 Schematic representation of a putative supraspinal 5-HT_{1A} autoreceptor pathway involving a 5-HT_{2C} receptor possibly located in the sacral region of the spinal cord in the rat (adapted from de Groat; 2002). WAY 100635 blocks inhibitory autoreceptors in the raphe neurones thus enhancing neuronal firing and increasing the release of 5-HT in the spinal cord. Spinal 5-HT activates excitatory 5-HT_{2C} receptors on inhibitory interneurones and suppresses parasympathetic preganglionic firing. The transmitter released by the inhibitory interneurones is still unidentified.

1.9 Dysfunctions of the lower urinary tract

Several conditions attributed to dysfunctions of the lower urinary tract exist, and these are a prevalent clinical problem for the health services in the western world due to the significant bother caused to the sufferers. Symptoms associated with lower urinary tract dysfunctions include bladder storage symptoms, sensation symptoms or voiding symptoms. Bladder storage symptoms include increased daytime frequency of voiding, nocturia, urgency and urinary incontinence. Bladder sensation symptoms include increased, reduced and absent bladder sensation whereas voiding symptoms include slow urinary stream, splitting or spraying of the urinary stream, intermittent urinary stream, hesitancy, straining to void, and terminal dribbling. The above symptoms are characteristic of the pathological conditions of the lower urinary tract, which include overactive bladder syndrome (OABS) affecting bladder function and stress urinary incontinence, urge urinary incontinence and mixed urinary incontinence affecting urethral function. Most of the conditions named above are prevalent in the female rather than the male population. Benign prostatic hyperplasia (BPH) is a common pathological condition affecting the male population.

1.9.1 Overactive bladder syndrome (OABS)

Initially, OABS was defined collectively as a condition encompassing bladder storage symptoms such as urgency, frequency, urge incontinence and nocturia which were thought to 'occur either singly or in combination' (Bates *et al.*, 1980). However, this definition of OABS was not completely representative of the condition, as individuals suffering a single symptom of the named symptoms above, were deemed as having OABS even though the underlying causes may not have been bladder related. Therefore, the International Continence Society (ICS) proposed a new definition of

OABS which was described as urinary urgency with or without urge incontinence, usually with frequency and nocturia, in the absence of infection or other obvious pathology (see Abrams *et al.*, 2002). This enabled the definition of OABS to be definitively characterised by functional disorders of the lower urinary tract. The pivotal symptom of OABS is urgency. Urgency is known to have two components: a quantitative component which identifies the episodes of urgency and can be counted and a qualitative component where the individual experiences a sudden desire to void with the inability to defer this desire (see Chapple *et al.*, 2005). The symptoms of OABS may be triggered by neurogenic etiology where conditions such as multiple sclerosis, stroke, diabetes, Alzheimer Disease, spinal stenosis and spinal cord injuries all present symptoms for OABS. It is well known that the suprapontine areas exert an inhibitory influence on the pontine micturition center, thus enabling urinary continence (Fall *et al.*, 1989; 1995). Damage to these areas reduces this inhibitory action thus leading to an overactive bladder. Damage to the spinal cord may also disrupt voluntary and suprasacral modulation of micturition thus leading to loss or changes in sensation of micturition events as well as sphincter coordination (see Mostwin, 2002). Myogenic etiology may be another contributing factor to OABS through disorders of smooth muscle tone. In some cases, bladder tissues from patients bear distinct features at the smooth muscle level thus leading to unstable contractions (see Moreland *et al.*, 2004). Loss of normal excitatory neural input increases signalling between smooth muscle cells thus leading to a state of overactivity. Smooth muscle function may also be impaired as a result of a partial urethral outlet obstruction.

1.9.2 Urinary incontinence (UI)

The ICS has defined UI as 'complaint of any involuntary leakage of urine' (see Abrams *et al.*, 2002). It is a disorder that affects women and men of all ages with a higher prevalence in women than men. An estimate of the prevalence of UI in women is that 1 in 4 women suffer from the condition, and this prevalence increases with age. Although not life threatening, many patients suffering UI affirm to a reduction in their quality of life, especially suffering social constraints due to fears of embarrassing situations in the public. Several types of UI have been described in the literature with the most common being stress urinary incontinence (SUI), urge urinary incontinence (UUI) and mixed urinary incontinence (MUI).

1.9.2.1 Stress urinary incontinence (SUI)

This is defined as the involuntary loss of urine which is associated with physical activities such as running, jumping and lifting, or with sneezing and coughing (see Abrams *et al.*, 2002). This is the most common type of urinary incontinence in women and is uncommon in men except after prostate surgery (Hunskaar *et al.*, 2000). Symptoms of SUI can be presented as either pure or mixed forms. Pure SUI occurs in women between the ages of 25 and 49 years, and decreases with increasing age, whereas mixed SUI (symptoms of both SUI and urge urinary incontinence [UUI]) increases with increasing age (Hannestad *et al.*, 2000). The main cause for SUI is insufficient urethral closure forces associated with displacement of the urethral wall e.g. after pregnancy, and this therefore leads to a low urethral closure pressure due to the intrinsic sphincter deficiency. Displacement of the bladder neck or urethra following a sudden increase in abdominal pressure, may also lead to SUI (Van der Vaart *et al.*, 2002; see Viktrup, 2002). These characteristics of SUI are observed in

pregnancy as well as pelvic trauma during vaginal delivery, and are therefore considered as risk factors contributing to the development of SUI. Other risk factors include obesity, constipation, smoking, chronic lung disease, neurological disorders, pelvic surgery, radiation and medications (see Viktrup, 2002).

1.9.2.2 Urge urinary incontinence (UUI)

This is defined as the complaint of involuntary leakage accompanied or immediately preceded by urgency (see Abrams *et al.*, 2002). UUI is mainly characterized by an overactive bladder. A distinction between UUI and SUI can be made following observations of involuntary detrusor contractions (IDCs) during the filling phase in cystometry (see Viktrup, 2002).

1.9.2.3 Mixed urge incontinence (MUI)

This has been defined by the ICS as the complaint of involuntary leakage associated with urgency as well as with exertion, effort, sneezing or coughing (see Abrams *et al.*, 2002) and can therefore be described as a combination of SUI and UUI.

1.9.3 Benign prostatic hyperplasia (BPH)

BPH is a chronic progressive condition primarily affecting the ageing male population. The prevalence of BPH symptoms is 26% in men aged 40 to 49 and 46% in men aged 70 and older (Wei *et al.*, 2004) with 90% of men demonstrating evidence of BPH by the age of 85 (McConnell *et al.*, 1994). BPH is characterised by abnormal and excessive non-malignant growth of the prostate tissue which over time starts impinging on the prostatic urethra and the bladder neck, leading to bladder outlet

obstruction. Bladder outlet obstruction leads to symptoms such as increased urinary frequency, urgency, nocturia, weak stream, hesitancy and straining whilst voiding.

Ligand	Receptor Subtype		
	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}
Agonists			
WAY 161503 [b] (2C)	-	-	8.40
Ro 60-0175 [a] (2C)	7.44	8.27	8.22
mCPP [a] (2C)	7.26	7.39	7.85
DOI [a] (2A)	9.03	7.55	8.08
BW723C86 [a] (2B)	7.20	7.33	7.11
Antagonists			
Mianserin [a] (2)	7.74	7.92	8.26
Ketanserin [a] (2A)	8.09	6.13	7.21
MDL 100907 [a] (2A)	8.73	5.99	7.52
RS 102221 [a] (2C)	5.54	5.95	8.30
SB 242084 [a] (2C)	6.07	6.84	8.15

a Knight *et al.*, 2004

b Cryan & Lucki, 2000

Table 1.1 pKi (log) values for the agonists and antagonists used in this study. The affinity values presented in this table are limited to the 5-HT_2 receptor family as this was the focus of this thesis.

1.10 Study aims

There is limited pharmacological success with respect to treatment of lower urinary tract dysfunctions specifically conditions such as SUI and OABS. However, it has been proposed that treatment of SUI should focus on increasing resistance of the striated urethral sphincter and urethral smooth muscle, whereas treatment of OABS should focus on improving bladder compliance, hence the proposal for the use of compounds selective for the 5-HT_2 receptor in this study. At present, there is very limited evidence regarding a role of 5-HT_2 receptors in micturition. The indication that 5-HT_2 receptors are involved in micturition came from a study by Steers and de Groat (1989) where they observed inhibitory effects of the non-selective 5-HT_2 receptor agonist mCPP on isovolumetric bladder contractions. Surprisingly, this inhibitory effect evoked by mCPP was blocked by the neuromuscular blocker pancuronium thus suggesting that this inhibition was related to somatic muscle contractions. Additionally, de Groat (2002) proposed that 5-HT_{2C} receptors may be physiologically involved in mediating the actions of the 5-HT_{1A} receptor. The aim of this thesis was therefore to investigate what role is played by 5-HT_2 receptors in micturition. The experiments in this thesis set out to address,

1) What role is played by 5-HT_2 receptors in bladder and urethral function in urethane anaesthetised female rats following bolus dosing of various selective 5-$HT_{2A/B/C}$ receptor agonists and antagonists?
2) What role is played by 5-HT_2 receptors in bladder and urethral function in urethane anaesthetised female rats following infusion dosing of 5-HT_{2C} receptor agonist Ro 60-0175 and 5-HT_{2C} and 5-HT_{2A} receptor antagonists SB 242084 and MDL 100907?

3) Are the effects evoked by the 5-HT_2 receptor agonists mediated centrally or peripherally?

Chapter 2

Methods

2 Methods

The experiments were carried out under the Animals (Scientific Procedures) Act, 1986. After completion of experiments, animals were killed by an overdose of pentobarbitone sodium (i.v.).

2.1 General preparation

Experiments were performed on 230 female Sprague-Dawley rats (200-300g). Anaesthesia was induced and maintained during initial surgery with isoflurane (4% in 100% oxygen which was reduced to 3% in 100% oxygen). The left jugular vein* was cannulated for anaesthetic and drug administration. The trachea was cannulated to maintain a patent airway. Isoflurane administration was then stopped and anaesthesia was maintained for the duration of the experiment with intravenous (i.v.) administration of urethane (1.2 g kg^{-1}, i.v.). Supplementary doses of urethane (0.1 g kg^{-1}, i.v.) were given as required. The right common carotid artery was cannulated with a heparinised cannula (20 units ml^{-1} heparin in 0.9% w v^{-1} saline) for the measurement of arterial blood pressure and sampling arterial blood gases for analysis. Blood pressure was measured using a pressure transducer (Gould Statham P23XL), and the heart rate (HR) derived electronically on-line from the blood pressure signal using *AcqKnowledge version 3.7.3* software (Biopac Systems Inc, USA). The depth of anaesthesia was assessed by the stability of blood pressure and heart rate, and by an absence of hindlimb withdrawal in response to paw pinch. Animals were spontaneously breathing oxygen enriched air (0.05 – 0.101 min^{-1}) supplied by use of a positive pressure pump (Harvard Rodent Ventilator 683). Body temperature was monitored by placing the rectal temperature probe underneath the animal and was maintained at 37-38 °C using a homeothermic blanket system (Harvard). Blood

samples were taken from the carotid arterial cannula and blood gases and pH were monitored with a Corning pH/blood gas analyser (Model 238). Blood gases were maintained between 90-130 mmHg Po_2, 40-50 mmHg Pco_2 and pH 7.3-7.4. Adjustments of the supplemented oxygen levels were made necessary to maintain blood gas and pH balance. The animals were infused (6 ml kg^{-1} h^{-1}, i.v.) with a solution comprising 10 ml plasma substitute (gelofusine), 10 ml distilled water, 0.04 g glucose and 0.168 g sodium bicarbonate, to prevent the development of non-respiratory acidosis and to maintain blood volume. The rats were placed in a stereotaxic frame and the head tilted approximately 10-15° to allow the animal to lie in a supine position to prevent the weight of the animal affecting the bladder and urethral pressure recordings.

* Some differences in general preparation were employed in experiments carried out at Pfizer (chapter 4). The left jugular vein was tri-cannulated. One cannula was used for the administration of urethane and the other two cannulae were used for the simultaneous infusions of agonist and vehicle or agonist and antagonist respectively. In these experiments, the animals spontaneously breathed room air and the plasma substitute solution was not infused. Body temperature was monitored by placing a small diameter rectal probe in the oesophagus. The right common carotid artery was cannulated for the measurement of arterial blood pressure and for withdrawal of blood samples for analysis of plasma concentration of the compounds. Additionally, for experiments carried out in this chapter, experiments were performed on the rats whilst lying on their backs.

2.1.1 Measurement of bladder pressure (cystometry method)

The ureters were exposed by retroperitoneal incisions and the proximal end of each ureter leading to the kidney was cannulated to prevent the bladder filling with urine during the experiments. The distal ends of the ureters were tied to ensure no backflow and hence leakage of saline from the bladder. The urinary bladder was exposed by a midline laparotomy. A small incision was made in the bladder dome and a single cuffed cannula (0.86 mm internal diameter (ID) and 1.52 mm outer diameter (OD)) was inserted into the bladder dome. The cannula was secured with a suture around the top of the bladder dome and this was followed by closure of the abdominal incision. The cuffed cannula was connected via a T-piece to a pressure transducer (Gould Statham P23XL) to record bladder pressure, and a syringe pump for the infusion of saline (0.9% wv^{-1}) at a rate of 0.1 ml min^{-1}, to evoke the micturition reflex. Backflow through this cannula allowed the bladder to be emptied of residual fluid after micturition had occurred (See Figure 2.1 for diagram showing a representation of the experimental method used).

For experiments carried out in chapter 4, a double lumen catheter was used and this was inserted into the bladder dome. The bladder catheter was constructed from polyethylene tubing (ID 1.4mm, OD 1.9mm) inside which two catheters were sealed. One catheter was connected to a pressure transducer and was used to record bladder pressure and to withdraw residual volume via a syringe on a plastic stopcock attached to the transducer. The second catheter was used for bladder infusions and was connected to a syringe infusion pump (Kd Scientific).

The bladder was filled to 80% volume of saline required to evoke a micturition reflex (calculated for each animal from the average bladder volume required to evoke the micturition response during three sequential cystometric investigations) prior to administering the compounds. This approach was adopted following initial experiments where no effects on EUS-EMG activity were observed following administration of the 5-HT_{2C} receptor agonist mCPP (300 $\mu g\ kg^{-1}$, i.v) in an empty bladder. It was therefore decided following discussions with colleagues that the bladder should be filled with 80% volume of saline required to evoke the micturition reflex and thereafter administer mCPP 5 minutes later. Hence, it appears that external urethral sphincteric activity controlled via a somatic output to the urethral striated muscle is dependant on bladder afferents activated by bladder distension.

2.1.2 External urethral sphincter (EUS) – Electromyography (EMG) recordings

Two fine copper wire electrodes (0.2 mm diameter) were used to measure the EMG of the external urethral sphincter. The tip of an electrode was positioned in the bevel of a needle (25G) and the needle was inserted percutaneously approximately 0.5 cm lateral and 0.5 cm caudal to the urethral orifice. The needle and wire were advanced approximately 0.5cm through the skin and the needle slowly withdrawn leaving the wire inserted in the external urethral sphincter. The second electrode was inserted in the same position on the contralateral side of the urethral orifice. The electrodes were connected to a Neurolog head stage (NL100; Digitimer, Welwyn Garden City, U.K.) and the signal was amplified (20 kHz; NL104) and filtered (500 Hz; NL125, and displayed on an oscilloscope (Tektronix, 2205).

2.1.3 Measurement of urethral pressure

A 1.4 F nylon catheter with a side-mounted microtip transducer located 1 mm from the catheter (SPR-671, Millar Instruments inc., Houston, Texas) was inserted into the urethra from the urethral orifice. Placement of the catheter in the mid region of the urethra exhibited high frequency oscillations in urethral pressure during urethral relaxation (in the voiding phase when the micturition reflex was tested), producing oscillations in urethral pressure (see figure 2.2). The microtip transducer catheter was connected to a pressure control unit (PCU-2000). Urethral pressure was measured as the force per unit area exerted by the wall of the organ (during contraction or relaxation) on the side mounted microtip transducer.

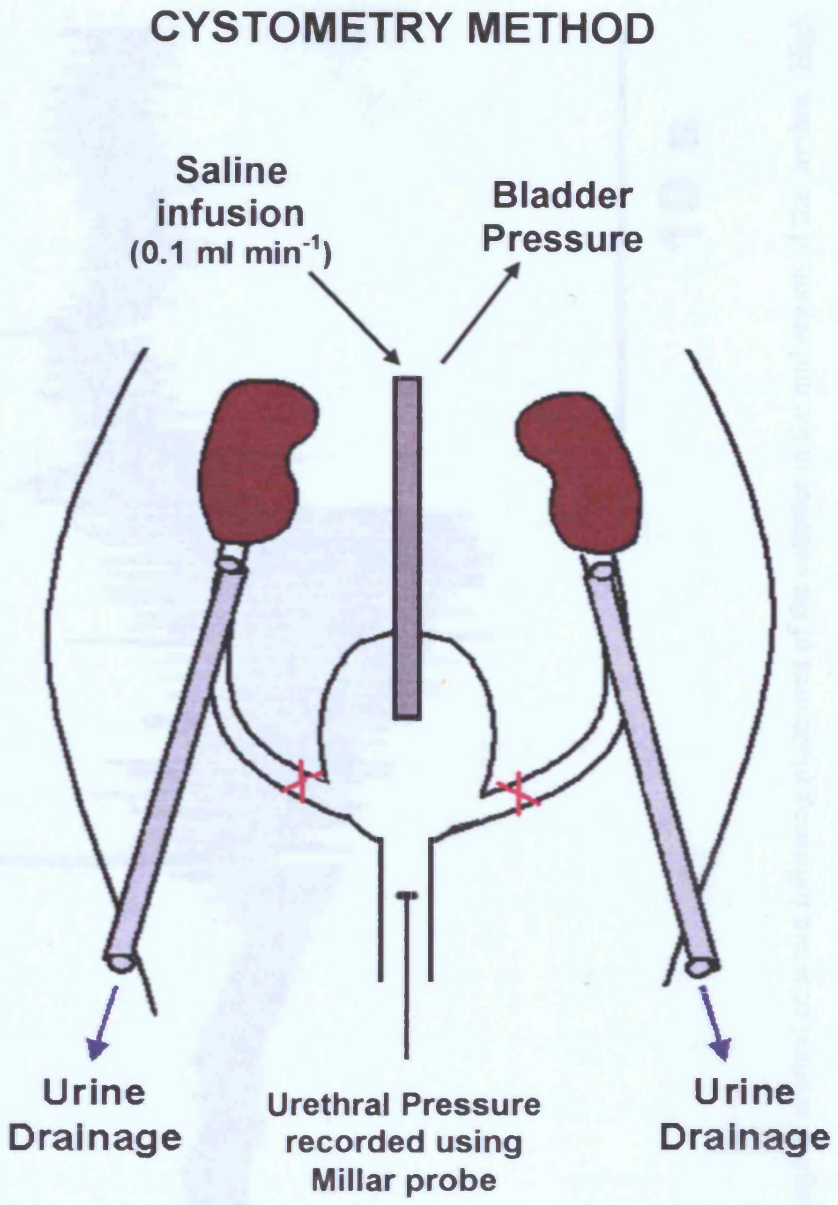

Figure 2.1 Schematic representation of the experimental method used. The ureters were tied and cut at the level of the bladder and cannulated at the level of the kidneys. A single* cuffed cannula connected via a T-piece to a pressure transducer and a syringe pump was inserted into the bladder dome. Electrodes used to record EUS-EMG activity were inserted on either side of the urethral orifice. The millar probe was inserted into the urethra from the urethral orifice.
* For experiments carried out in chapter 4, a double lumen cuffed cannula was inserted into the bladder dome.

Adapted from Wibberley *et al.*, 2002.

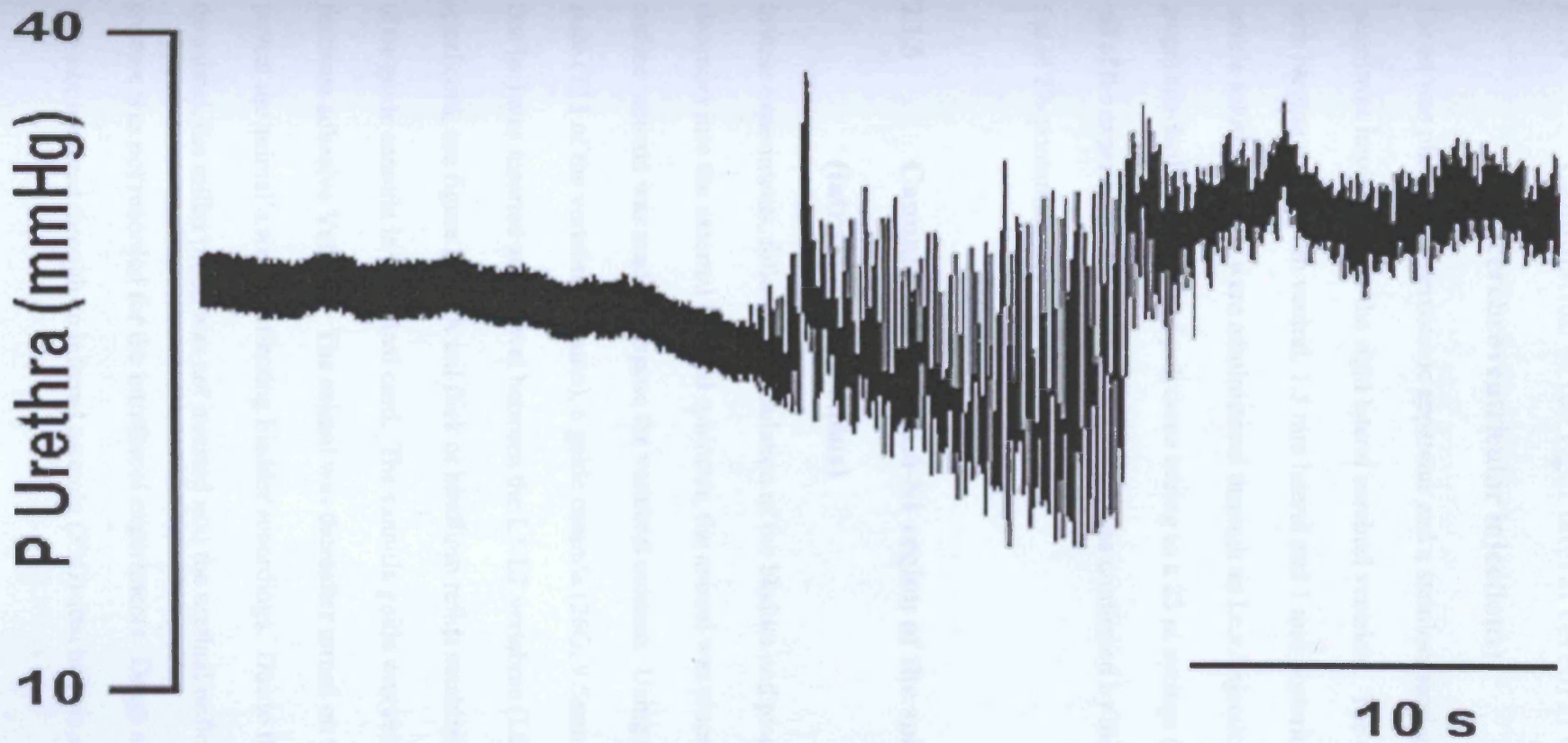

Figure 2.2 A representative trace of recordings of urethral pressure following placement of the catheter in the mid region of the urethra. High frequency bursts pressure oscillations during urethral relaxation on voiding were observed.

2.1.4 Cannulation of the right lateral cerebral ventricle (intracerebroventricular injections)

The rat was placed in a stereotaxic apparatus and a stainless steel guide cannula (22 gauge) was implanted into the right lateral cerebral ventricle. The coordinates used from bregma were 4 mm ventral, 1.5 mm lateral and 1 mm posterior. Drugs and vehicle solutions (5 μl) were administered through an i.c.v. injection cannula (28 gauge) attached by a length of polythene tubing to a 25 μl syringe (Hamilton). At the end of the experiment, the cannula placement was confirmed by the administration of 5 μl of 2% pontamine sky blue dye.

2.1.5 Cannulation of the L6-S1 region of the spinal cord (intrathecal injections)

In these experiments, following cannulation of the bladder and placement of the EMG electrodes into the external urethral sphincter, the animal was placed on its front and a midline incision was made to expose the vertebral column. Using the last rib as a guide (T13 of the vertebral column), a guide cannula (26G, 9.5mm in length; Plastics One Inc) was inserted at the level between the L1-L2 vertebrae (L6-S1 region of the spinal cord; see figure 2.3). A tail flick or hindlimb reflex established the placement of the guide cannula in the spinal cord. The cannula guide was secured in place using the tissue adhesive Vetbond. The animal was thereafter turned on to its right side to prevent the animal's weight affecting bladder recordings. Due to the positioning of the animal, the millar probe was not inserted into the urethral orifice and thus urethral pressure was not recorded for the intrathecal experiments. Drugs and vehicle (10 μl) were administered through an internal cannula (33G) attached via a length of

polythene tubing to a 50 µl syringe (Hamilton). The location of the cannula placement for the injection site was subsequently confirmed at the end of the experiment by administering 10 µl of pontamine sky blue dye and performing a laminectomy thereafter.

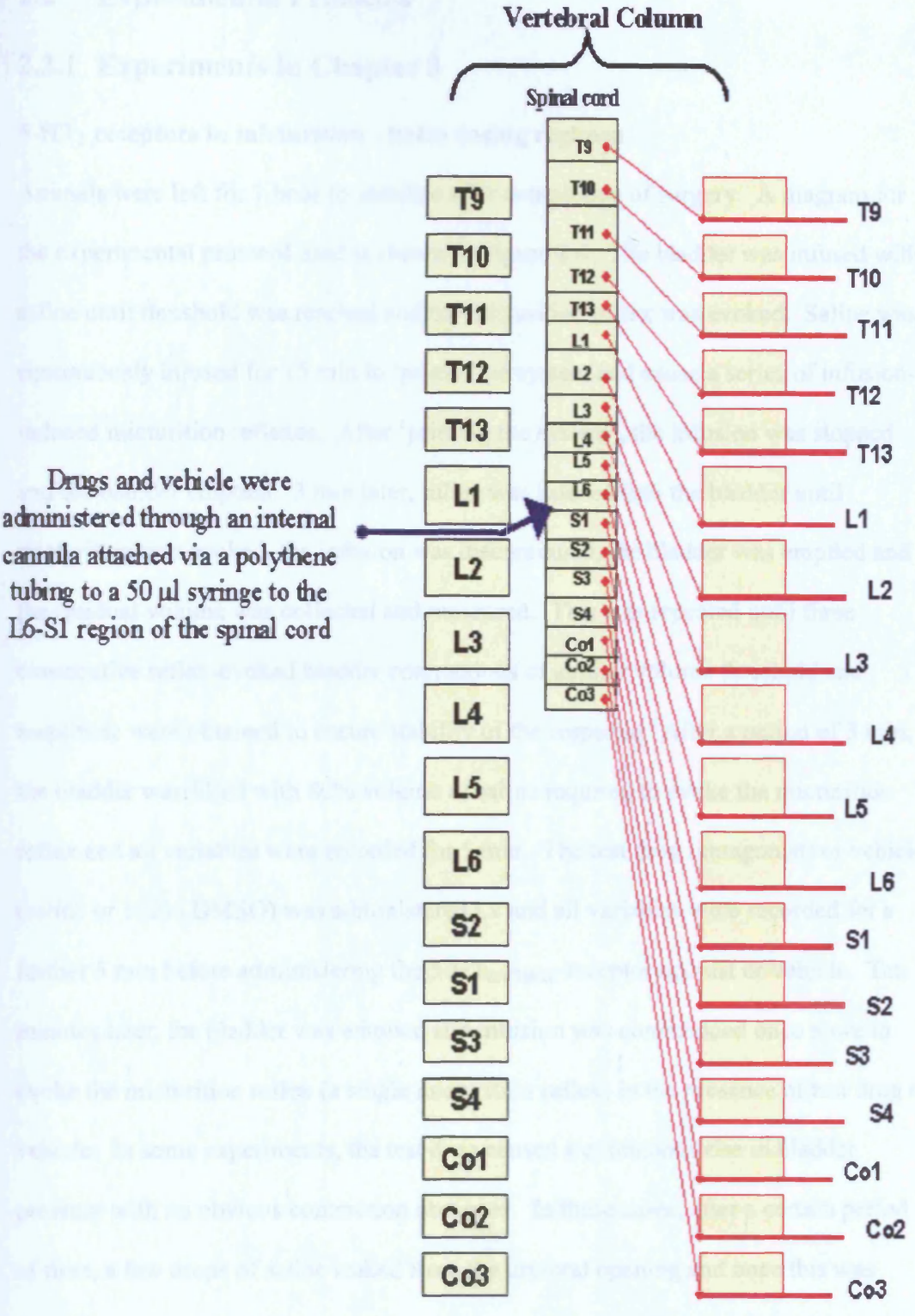

Figure 2.3 Schematic representation of the spinal cord. Histochemical studies have confirmed that autonomic nuclei in the lumbosacral spinal cord innervate the lower urinary tract. Additionally, it has also been shown that receptors (specifically 5-HT_{1A}) in the L6/S1 region of the spinal cord have an important physiological role in the tonic control of the descending limb of the micturition pathway. This region of the spinal cord was therefore chosen in this study to investigate whether 5-HT_2 receptors are also physiologically involved in the control of the micturition pathway. Adapted from Ziegler, 1988.

2.2 Experimental Protocols

2.2.1 Experiments in Chapter 3

5-HT_2 receptors in micturition - bolus dosing regimen

Animals were left for 1 hour to stabilise after completion of surgery. A diagram for the experimental protocol used is shown in Figure 2.4. The bladder was infused with saline until threshold was reached and the micturition reflex was evoked. Saline was continuously infused for 15 min to 'prime' the system and cause a series of infusion-induced micturition reflexes. After 'priming the system', the infusion was stopped and the bladder emptied. 3 min later, saline was infused into the bladder until micturition was evoked, the infusion was discontinued, the bladder was emptied and the residual volume was collected and measured. This was repeated until three consecutive reflex-evoked bladder contractions of similar volume threshold and amplitude were obtained to ensure stability of the response. After a period of 3 min, the bladder was filled with 80% volume of saline required to evoke the micturition reflex and all variables were recorded for 5 min. The test drug (antagonist) or vehicle (saline or 100% DMSO) was administered i.v and all variables were recorded for a further 5 min before administering the 5-$HT_{2A/2B/2C}$ receptor agonist or vehicle. Ten minutes later, the bladder was emptied and infusion was commenced once more to evoke the micturition reflex (a single micturition reflex) in the presence of test drug or vehicle. In some experiments, the test drug caused a continuous rise in bladder pressure with no obvious contraction observed. In these cases, after a certain period of time, a few drops of saline leaked from the urethral opening and once this was observed, the infusion was switched off. These responses were taken to indicate that the micturition response had been abolished.

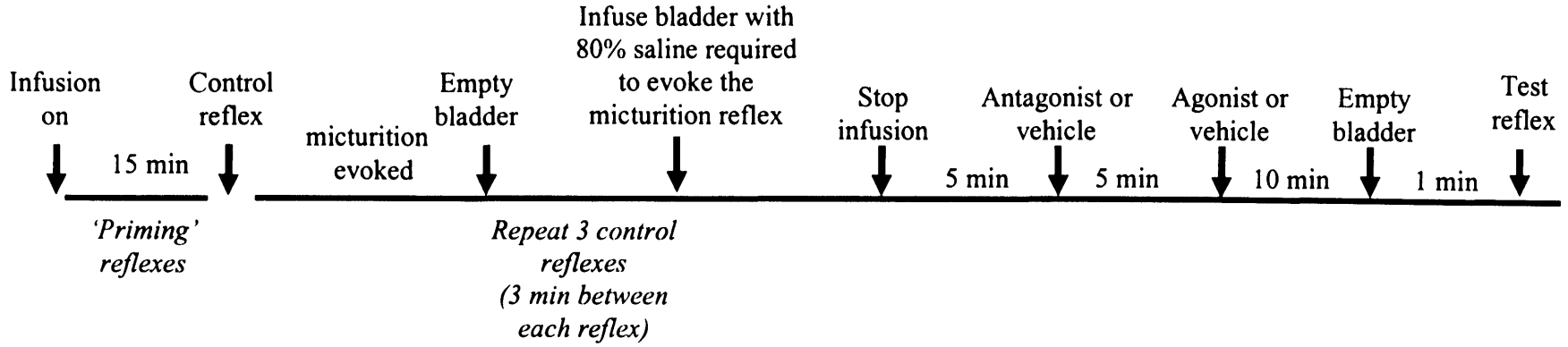

Figure 2.4 Diagram showing the experimental protocol used for the bolus dosing regimen. It should be noted that there is a 1 hour stabilization period following surgery.

2.2.2 Experiments in Chapter 4

5-HT_2 receptors in micturition - infusion dosing regimen

Figure 2.5 represents the protocol used for the infusion dosing regimen. Following attainment of three consecutive reflex-evoked bladder contractions of similar amplitude, the bladder was filled with 80% volume of saline required to evoke the micturition reflex, and all variables recorded for 5 min. Baseline values were recorded for EUS-EMG activity, urethral pressure and blood pressure during this period. The bladder was emptied and 2 min later a micturition reflex was evoked. Three minutes later, test drugs or vehicle were infused into the animal in total volume of 100 $\mu l\ kg^{-1}\ min^{-1}$ for agonist alone or agonist plus antagonist. Thirty seconds later infusion of saline into the bladder was commenced until 80% volume of saline required to evoke the micturition reflex was achieved. All variables were recorded for a further 5 min. The bladder was thereafter emptied and 2 min later saline was infused into the bladder to evoke a micturition reflex in the presence of test compound or vehicle. Subsequently, a blood sample was withdrawn from the common carotid artery for analysis of the plasma concentrations of the test compounds. 3 min later, the bladder was again filled with 80% volume of saline required to evoke the micturition reflex and all variables recorded for a further 5 min. A second micturition reflex was tested and a second blood sample (0.2 ml) was withdrawn from the animal (see subsequent paragraph for details on test drug infusions). This was repeated until a total of 6 micturition reflexes and 6 blood samples were obtained (in the presence of vehicle and test compounds) for the duration of each experiment. The compounds or vehicles were infused continuously into the animal for a duration of approximately 90 min.

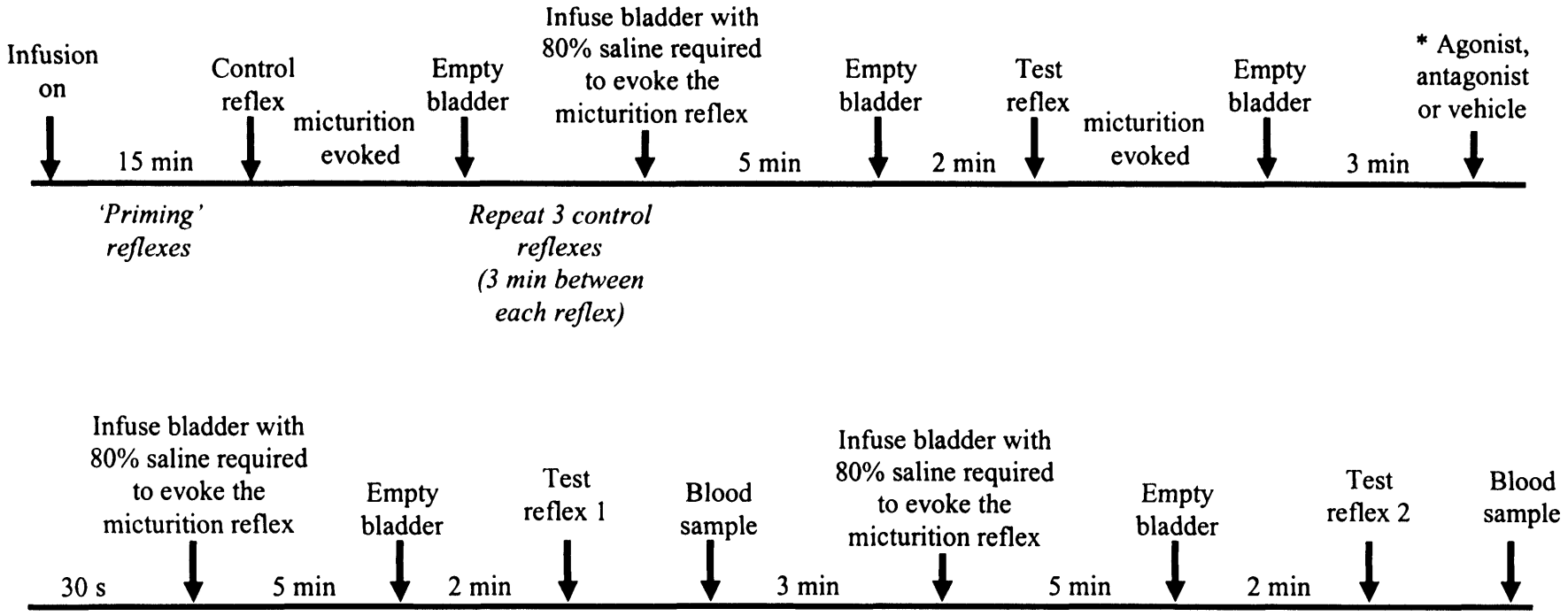

Figure 2.5 Diagram showing the experimental protocol used for the infusion dosing regimen. It should be noted that there is 1 hour stabilization period after surgery.

* Cumulative dosing was utilized for the agonist infusion where three different doses of the compound were infused (see below) for approximately 30 min each dose.

The compounds under investigation using the infusion dosing regimen were 5-HT_{2C} receptor agonist Ro 60-0175 and 5-HT_{2C} and 5-HT_{2A} receptor antagonists SB 242084 and MDL 100907 respectively.

Ro 60-0175 was administered for a duration of approximately 30 min as i.v. infusions of 27, 90 and 270 μg kg^{-1} min^{-1} targeting 10, 30 and 100 nM free plasma concentration in each rat respectively (see Figure 2.6 showing the cumulative target concentrations calculated for Ro 60-0175). As Ro 60-0175 is a selective 5-HT_{2C} receptor agonist (Ki = 1nM) from cloned rat binding studies (Hemrick-Luecke & Evans, 2002), it was decided following in-house discussions at Pfizer with colleagues that 10, 30 and 100 x Ki nM (free plasma concentration) would be suitable to investigate the selectivity and efficacy of Ro 60-0175 in this study. Therefore, following in-house pharmacokinetic/pharmacodynamic (PK/PD) studies in the rat and *in vitro* plasma protein binding (PPB), carried out at Pfizer (Sandwich, UK), cumulative infusion doses were simulated, where the low dose of Ro 60-0175 infused at a rate of 27μg kg^{-1} min^{-1} (targeting 10 nM free plasma concentration) was proposed to be selective for 5-HT_{2C} receptors and the high dose infused at a rate of 270 μg kg^{-1} min^{-1} (targeting 100 nM free plasma concentration) was proposed to be non-selective, thus activating other receptors including 5-HT_{2A}. In this study, the duration of infusion of each dose of Ro 60-0175 was designed to fit in with the cystometry experimental protocol described above.

SB 242084 is an antagonist with high affinity for the 5-HT_{2C} receptor subtype where Ki = 0.94 (~1 nM) at the human 5-HT_{2C} receptor (Gleason *et al.*, 2001). It was established that targeting 3 x Ki (3 nM) plasma concentration would be selective for

the 5-HT_{2C} receptor. In order to obtain the desired free plasma levels of SB 242084, calculations performed by Pfizer Drug Metabolism (PDM) using PK/PD studies and PPB determination carried out in-house, concluded that SB 242084 was to be administered as a loading dose of 22.5 $\mu g\ kg^{-1}\ min^{-1}$ for 15 min, and thereafter maintained at a dose of 3.5 $\mu g\ kg^{-1}\ min^{-1}$ for the remaining duration of the experiment (see Figure 2.7 for simulation graph of target concentration for SB 242084).

MDL 100907 is an antagonist with a high selectivity for the 5-HT_{2A} receptor where Ki = 0.78 (~1 nM) at the human 5-HT_{2A} receptor (Gleason *et al.*, 2001). Similar to SB 242084 above, it was established that targeting 3 x Ki (3 nM) would be an effective free plasma concentration of MDL 100907, as this would be selective for the 5-HT_{2A} receptors. In order to obtain the desired free plasma levels of MDL 100907, calculations performed by PDM using PK/PD studies and PPB determination carried out in-house, concluded that MDL 100907 was to be administered as a loading dose of 16.7 $\mu g\ kg^{-1}\ min^{-1}$ for 15 min, and thereafter maintained at a dose of 0.83 $\mu g\ kg^{-1}\ min^{-1}$ for the remaining duration of the experiment (see Figure 2.8 for simulation graph of target concentration for MDL 100907).

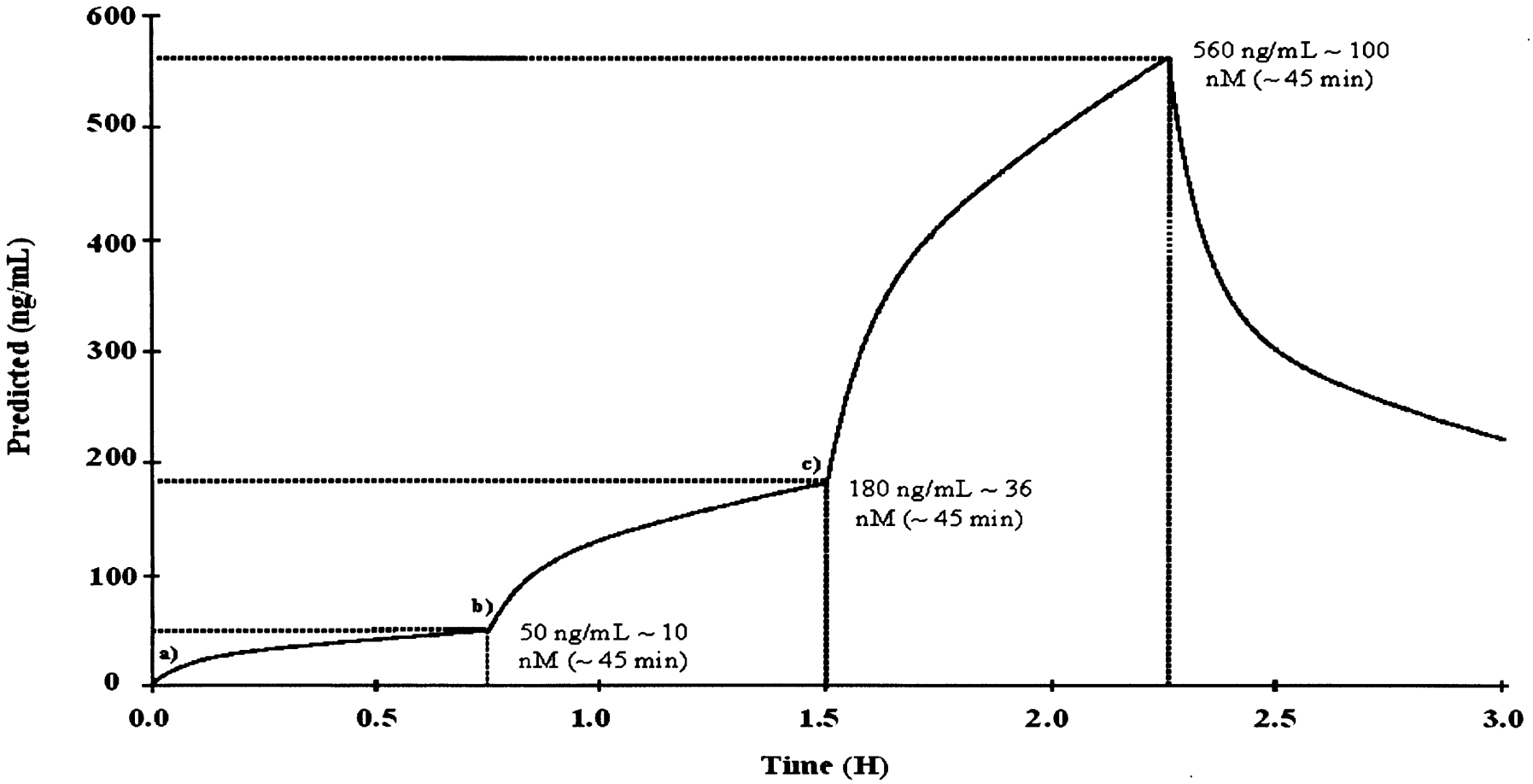

Figure 2.6 Simulation graph showing cumulative predicted plasma concentrations calculated for Ro 60-0175 (courtesy of PDM; Pfizer, Sandwich UK). To achieve target concentrations of 10, 30 and 100 nM for Ro 60-0175, the compound was infused at 3 cumulative doses of **a)** 27 μg kg^{-1} min^{-1} (~10 nM), **b)** 90 μg kg^{-1} min^{-1} (~30 nM) and **c)** 270 μg kg^{-1} min^{-1} (~100 nM). The duration of infusion of each dose of Ro 60-0175 was designed to fit in with the cystometry experimental protocol. Simulations were made using previous PK/PD studies in the rat carried out in-house for Ro 60-0175.

To convert the predicted concentration of Ro 60-0175 from ng/mL (see Figure 2.6 above) to Free nM, the following formula was used:

$$\frac{\text{ng/mL} \times 1000}{\text{relative molecular mass (Mr)}} \times \text{Free unbound plasma (FUpl)} = \text{nM Free}$$

Relative molecular mass:	226.7
FUpl obtained from rat plasma protein binding studies:	0.04

* The above calculations, were performed courtesy of colleagues in PDM at Pfizer (Sandwich, UK)

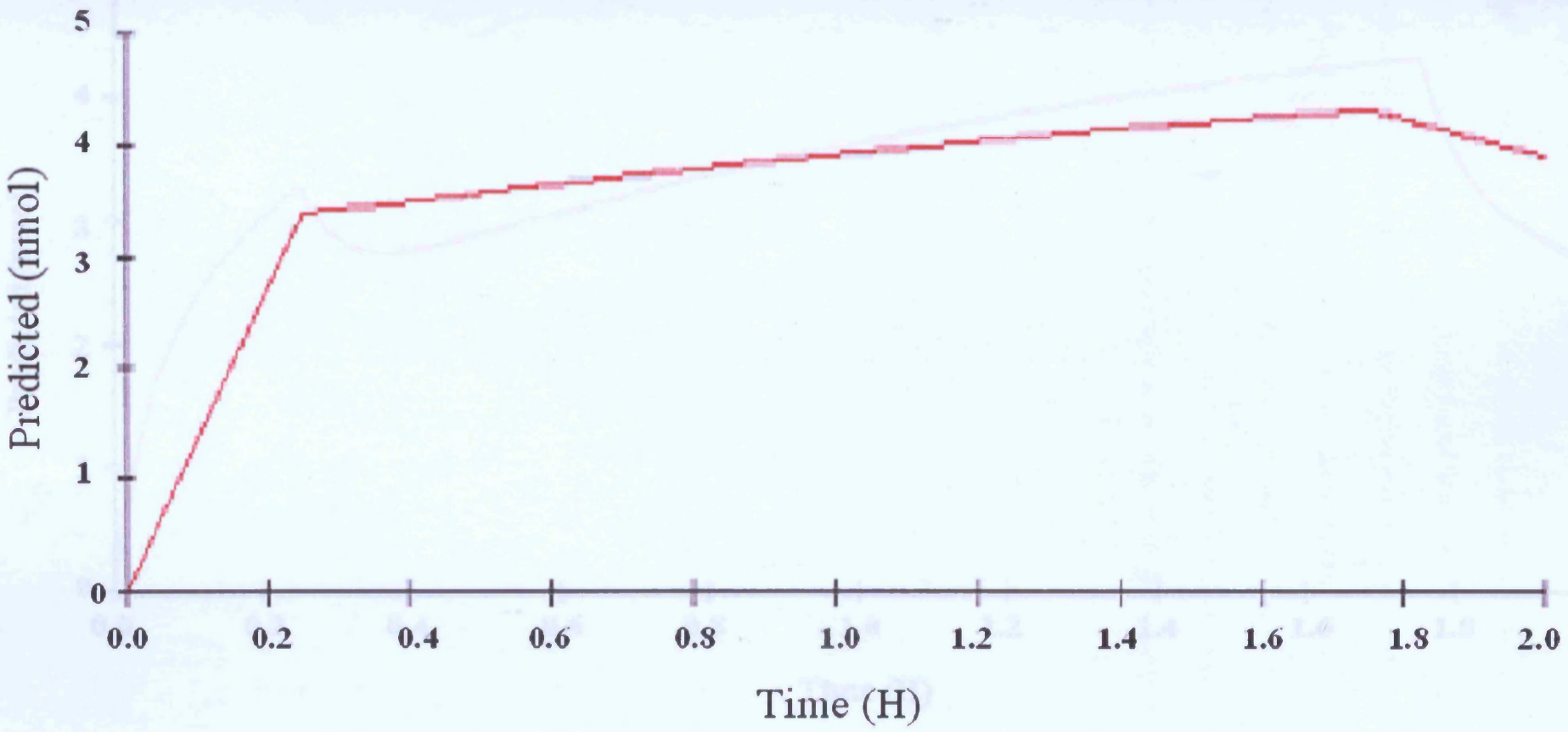

Figure 2.7 Simulation graph showing the free plasma target concentration calculated for SB 242084 (courtesy of PDM; Pfizer, Sandwich UK). To achieve a target concentration of 3nM (3 x Ki) for SB 242084, the compound was initially infused as a loading dose at a rate of 22.5 $\mu g\ kg^{-1}\ min^{-1}$ for 15 minutes and thereafter maintained at a rate of 3.5 $\mu g\ kg^{-1}\ min^{-1}$ for the remaining duration of the experiment. The duration of infusion of both the loading dose and maintenance dose of SB 242084 was designed to fit in with the cystometry experimental protocol. Simulations were made using previous PK/PD studies in the rat carried out in-house for SB 242084.

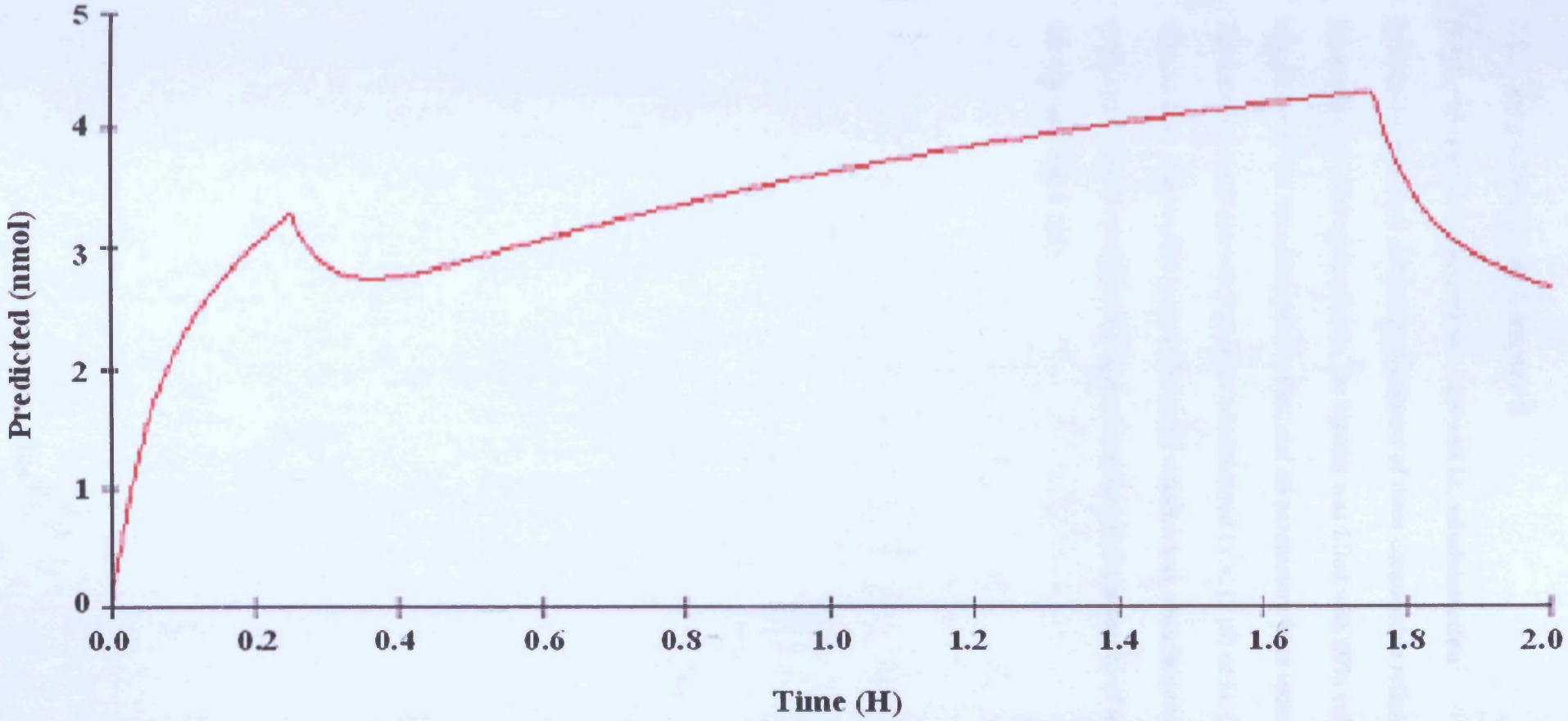

Figure 2.8 Simulation graph showing the infusion rate calculated for MDL 100907 (courtesy of PDM; Pfizer, Sandwich UK). To achieve a target concentration of 3nM (3 x Ki) for MDL 100907, the compound was initially infused as a loading dose at a rate of 16.7 μg kg^{-1} min^{-1} for 15 minutes and thereafter maintained at a rate of 0.83 μg kg^{-1} min^{-1} for the remaining duration of the experiment. The duration of infusion of both the loading dose and maintenance dose of MDL 100907 was designed to fit in with the cystometry experimental protocol. Simulations were made using previous PK/PD studies in the rat carried out in-house for MDL 100907.

2.2.3 Experiments in Chapter 5

5-HT_2 receptors in micturition - i.c.v and i.t. administration

In these experiments, following attainment of three consecutive reflex-evoked bladder contractions of similar amplitude, the bladder was filled with 80% volume of saline required to evoke the micturition reflex, and all parameters were recorded for 5 min. The agonist or vehicle was thereafter administered i.c.v. (5 µl) or i.t. (10 µl). Ten minutes later, the bladder was emptied and infusion was commenced once more to evoke the micturition reflex (a single micturition) in the presence of test drugs or vehicle (see Figure 2.9).

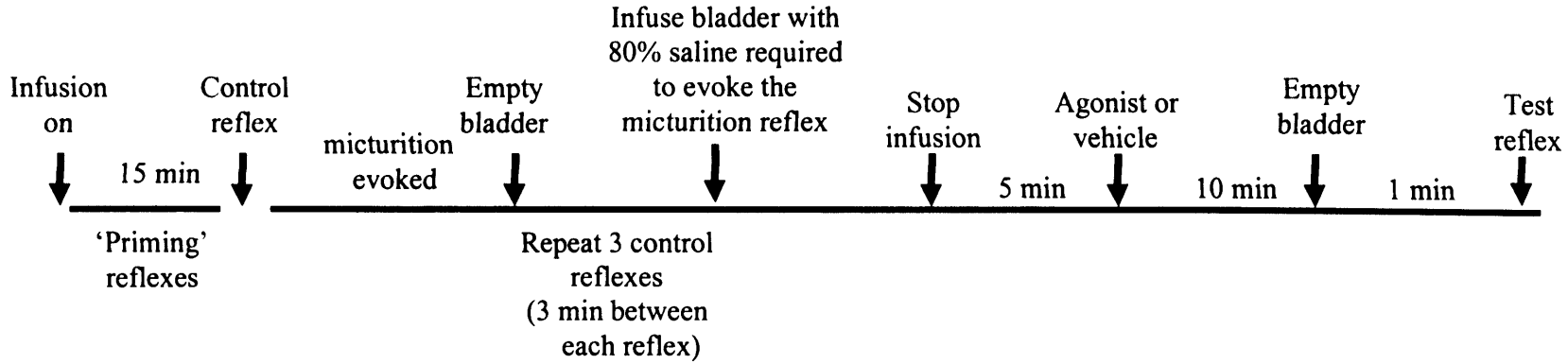

Figure 2.9 Diagram showing the experimental protocol used for the i.c.v. and i.t. dosing regimen. It should be noted that there is 1 hour stabilization period after surgery.

2.3 Data capture

Arterial blood and bladder pressure were continuously displayed on a chart recorder (Grass Instruments) and captured (2000 samples per second) by a MP 100 WSW interface (Biopac Systems Inc, USA) to allow data to be acquired and analysed offline using *Acknowledge version 3.7.3* software (Biopac Systems Inc, USA). Heart rates (HR) were derived electronically on-line from the blood pressure signal using the Biopac system. The amplified raw EMG signal was captured (2000 samples per second) and the input integrated off-line using *Acknowledge version 3.7.3* software (Biopac Systems Inc, USA). For EUS-EMG activity, the noise levels were verified at the end of the experiment by slowly administering the neuromuscular blocker decamethonium bromide (3 mg kg^{-1}, i.v.) to abolish activity. The background noise was then determined and subtracted from the measured values. Urethral pressure was captured (500 samples per second) and displayed using the *Acknowledge version 3.7.3* software and analysed offline.

For experiments carried out in chapter 4, blood pressure, bladder pressure, urethral pressure, raw and integrated EUS-EMG were recorded onto a computer hard disk using a CED (Cambridge Electronic Design) 1404 + interface and Spike 2 (version 5.06) data collection software which was used for off-line analysis. HR was derived off-line from the blood pressure signal using the Spike system. Additionally, due to Home Office restrictions at Pfizer (Sandwich, UK), no decamethonium was administered for experiments carried out in the establishment but rather change in EUS-EMG signal was calculated from subtracting baseline EUS-EMG activity pre-treatment from EUS-EMG activity post-treatment and percentage changes in EUS-EMG signal calculated thereafter.

2.4 Analysis of results

Saline infusion into the bladder evoked large-amplitude bladder contractions with corresponding urethral relaxations and bursts of the EUS-EMG each of which represents a micturition reflex (Maggi *et al.*, 1986; see Figure 2.10). The following variables were measured on the micturition reflex; volume threshold (ml) - defined as the volume of saline required to elicit voiding, pressure threshold (mmHg) – the bladder pressure recorded at the start of a micturition contraction, and residual volume - expressed as a percentage of the total volume infused (see Figures 2.10 and 2.11). As mentioned in the previous section, background noise was subtracted from the measured integrated EUS-EMG activity. The total integrated EUS-EMG activity using the root mean square of the raw EUS-EMG was used for analysis as it produced waveforms that were more easily analyzable than the noisy raw EMG signal. Baseline values of the area under the curve of the rectified EUS-EMG signal were analysed over a 1 min period before administration of test drug or vehicle and for 5 min after drug/vehicle administration. It should be noted that these baseline variables were recorded whilst the bladder was filled with 80% volume of saline required to elicit a micturition reflex. The percentage change of the total integrated EUS-EMG signal following deduction of background noise was calculated thereafter. Similarly, the mean baseline variables for urethral pressure, blood pressure and heart rate were also measured over a 1 min period before addition of test drug/vehicle with post-treatment effects measured for 5 min thereafter.

For experiments carried out in chapter 4, raw EUS-EMG activity was rectified and smoothed (0.1s time constant) using Spike 2 functions. The resulting baseline EUS-EMG activity was analysed as described in the paragraph above.

2.5 Statistical analysis

Changes in all the above variables were measured after drug administration and all values are expressed as Δ mean (%) ± s.e.mean or Δ mean ± s.e.mean. Drug evoked changes on the micturition reflex were compared to control reflexes (calculated mean of the variables measured for the 3 control micturition reflexes) using unpaired Student's t-test. Changes in mean integrated EUS-EMG activity, urethral pressure, blood pressure and heart rate caused by the test drugs were compared with time matched vehicle controls (*), antagonist + agonist (#) or antagonist alone (+) using two-way analysis of variance and the least significant difference test (Sokal & Rohlf, 1969). Values of $P<0.05$ were considered to be statistically significant for all the statistical tests performed.

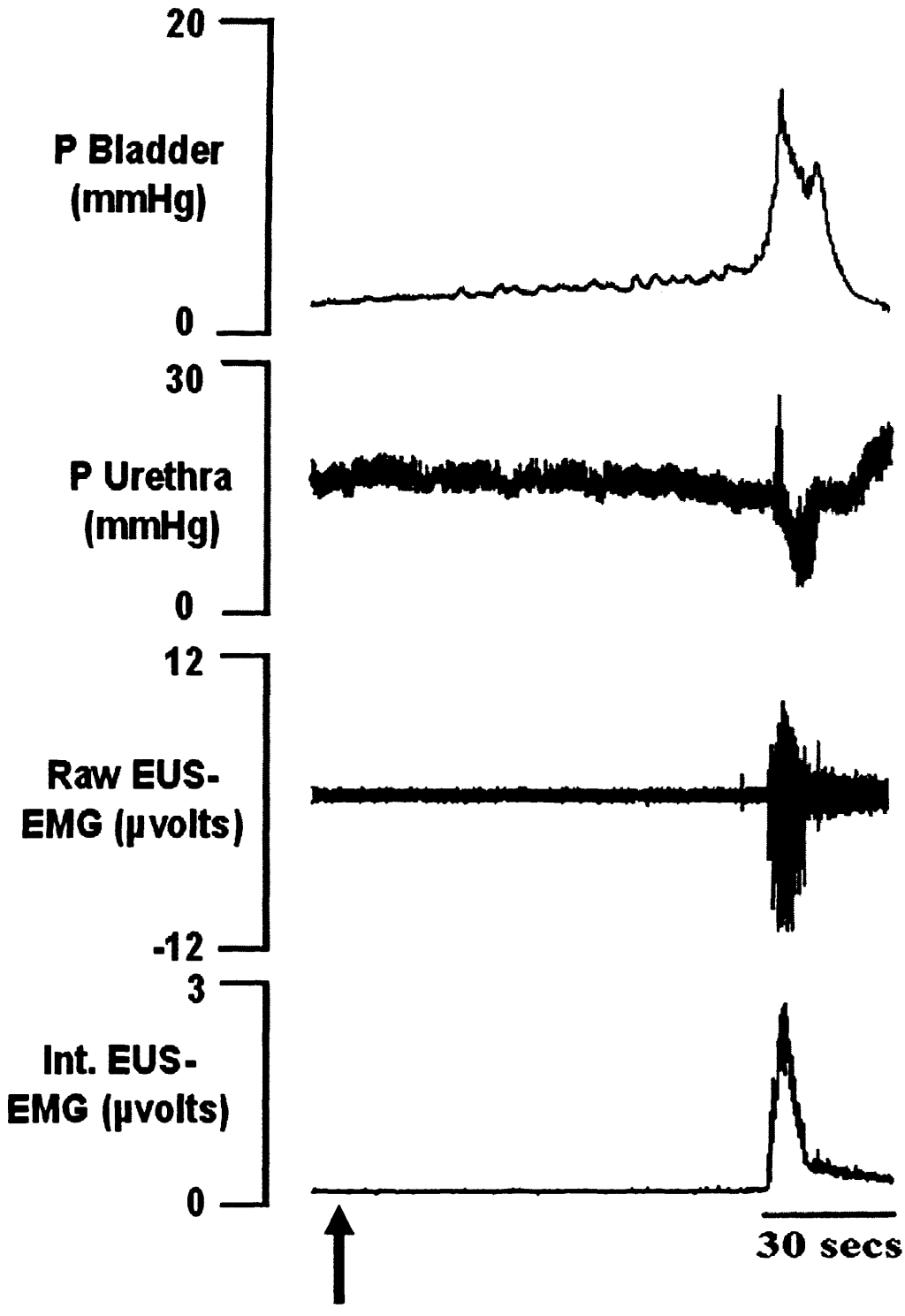

Figure 2.10 Urethane anaesthetised female rats: traces showing changes in bladder and urethral pressures, raw and integrated urethral striated muscle (EUS-EMG activity) during intravesical infusion of saline into the bladder (the distension evoked micturition reflex). Arrow denotes onset of saline infusion into the bladder.

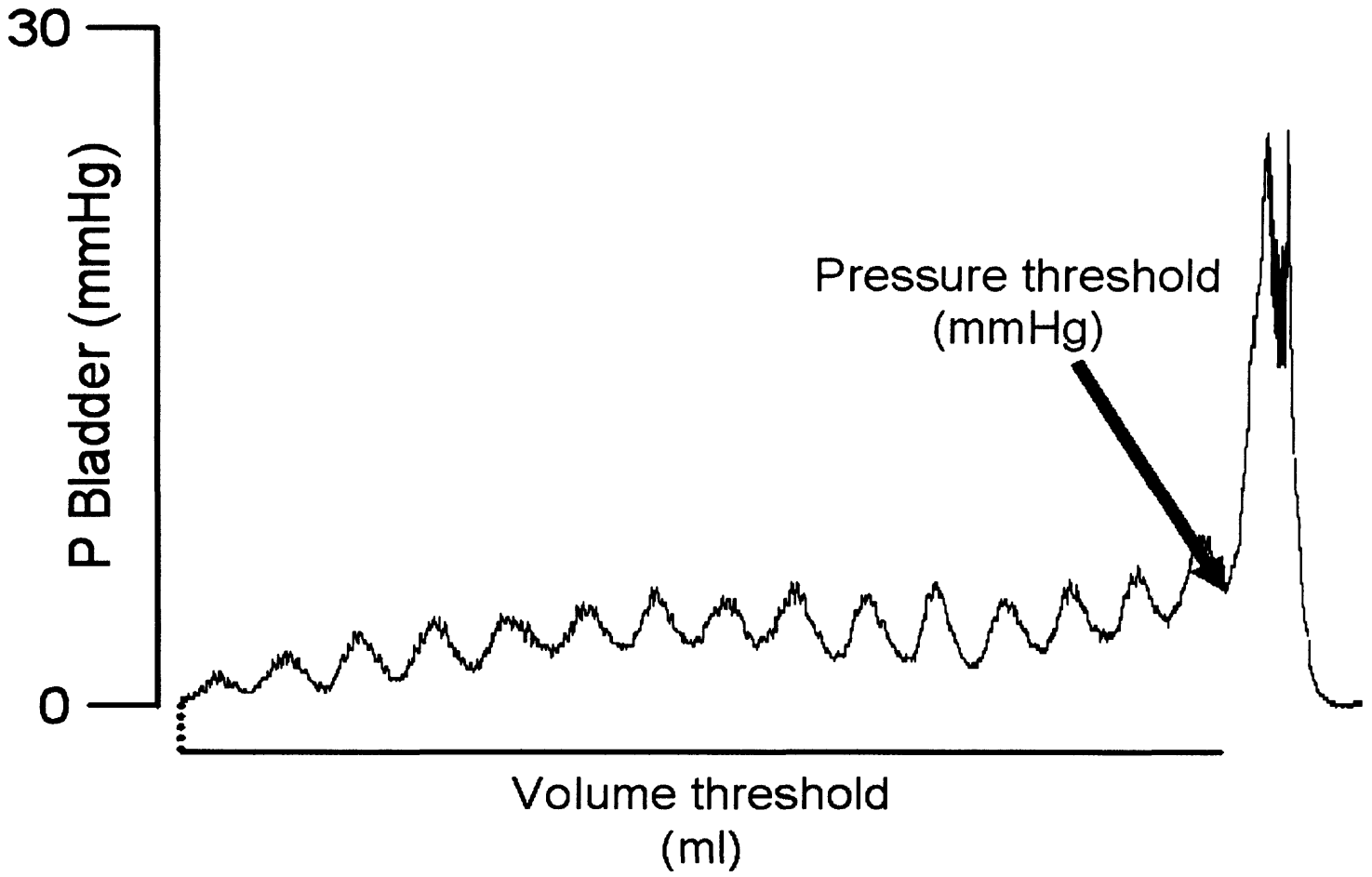

Figure 2.11 Rat cystometry method, trace showing distension evoked micturition reflex and the measurements taken.

2.6 Drugs and solutions

Drugs and chemicals were obtained from the following sources: 8,9-Dicloro-2,3,4,4a-tertrahydro-1H-pyrazinol[1,2,-α]quinoxaline-5(6*H*)-one hydrochloride (WAY-161503), (αS)-6-Chloro-5-fluoro-α-methyl-1H-indole-1-ethanamine fumarate (Ro 60-0175), α-methyl-5-(2-thienylmethoxy)-1 H-indole-3-ethanamine (BW 723C86), ketanserin, mianserin and 8-[5-(5-amino 2,4-dimethoxyphenyl) 5-oxopentyl]-1,3,8-triazaspiro[4,5] decane-2,4-dione (RS 102221) from Tocris Cookson Ltd., Avonmouth, Bristol, U.K; urethane, decamethonium bromide, 1-(3-Chlorophenyl) piperazine hydrochloride (mCPP) and (2,5-dimethoxy-4-idophenyl)-2-aminopropane hydrochloride (DOI) from Sigma Aldrich Chemicals, Poole , Dorset, U.K; isoflurane from Abbots Labs, Queenborough, Kent, U.K; gelofusine from Braun Medical Ltd, Aylesbury, Bucks, U.K; sodium chloride, glucose, sodium bicarbonate from Merck/BDH, Poole, Dorset, U.K; heparin from CP Pharmaceuticals from CP Pharmaceuticals Ltd, Wrexham, U.K. 2-amino-4-(4-fluoronaphth-1-yl)-6-isopropylpyrimidine (RS 127445) and 6-Chloro-2,3-dihydro-5-methyl-N-[6-[(2-methyl-3-pyridinyl)oxy]-3-pyridinyl]-1H-indole-1-carboxyamide dihydrochloride 6-Chloro-5-methyl-1-[[2-(2-methylpyrid-3-yloxy)pyrid-5-yl]carbamoyl]indoline dihydrochloride (SB 242084) were a gift from Pfizer Global Research and Development, Sandwich, Kent, UK; α-anilino-N-2-m-chlorophenoxypropylacetamide (BW501C67) was a gift from Wellcome Research Laboratories, Beckenham, Kent; and R-(+)-a- (2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidinem ethanol (MDL 100907) was a gift from Merrill Dow, Cincinnati, Ohio. mCPP, WAY-161503, Ro 60-0175, DOI, BW723C86 and BW501C67 were dissolved in 0.9% w v^{-1} saline. Mianserin, ketanserin, * MDL 100907, RS 102221, * SB 242084 and RS 127445 were dissolved in 100% DMSO for i.v. bolus doses. All i.v. bolus

doses were administered in a 0.1 ml volume followed by a flush of 0.1 ml saline. All i.c.v. doses were administered in a 5 µl volume over a 20 s period whereas all i.t. doses were administered in a 10 µl volume over the same time period. All drugs were given as their salts.

* For i.v. infusion studies (chapter 4), MDL 100907 and SB 242084 were dissolved in a mixture comprising 50% cremaphor, 40% tetraglycol and 10% ethanol (CTE) to give a final concentration of 4% of the mixture when made up to the required volume in saline.

Chapter 3

Effects of i.v. bolus doses of 5-HT_2 receptor agonists and antagonists on the urethra and the micturition reflex

3.1 Introduction

It has been established that central 5-HT containing pathways play an important role in the control of micturition (see Ramage, 2006). The major receptor subtypes that have been identified to play a role in this pathway are 5-HT_{1A} (Lecci *et al.*, 1992; Testa *et al.*, 1999; Conley *et al.*, 2001) at both supraspinal and spinal levels (Kakizaki *et al.*, 2001; Secker *et al.*, 2003; Yoshiyama *et al.*, 2003) and 5-HT_7 receptors which act only at the supraspinal level (Read *et al.*, 2003). Interestingly, data from these experiments would imply that 5-HT containing neurones play an excitatory role in the control of micturition. This contradicts the established view that 5-HT pathways are inhibitory. To get round this contradiction, a 5-HT_{2C} receptor pathway has been proposed, which excites inhibitory interneurones controlling the parasympathetic preganglionic neurones innervating the bladder (see de Groat, 2002). This would indicate that activation of 5-HT_{2C} receptors would cause inhibition of the micturition reflex as reported by Steers and de Groat (1989) using the so called archetypical 5-HT_{2C} receptor agonist mCPP which is fairly non selective between the 5-HT_2 receptor subtypes (see Barnes & Sharp, 1999). Further, this hypothesis would imply that 5-HT_{2C} receptor antagonists would have an excitatory action on the micturition reflex. However, this has not yet been examined although the selective 5-HT_{2A} receptor antagonist ketanserin (see Barnes & Sharp, 1999) has been reported to inhibit isovolumetric bladder contractions (Testa *et al.*, 2001). Further, in the paper by Steers and de Groat (1989), it was also suggested that 5-HT_{2C} receptor activates "somatic muscle contraction" i.e. the EUS. In this respect, in the cat, 5-HT_2 receptors have also been implicated in the control of the EUS (Danuser & Thor, 1996).

The aim of the present study was therefore to investigate the role of 5-HT_2 receptor subtypes in the control of micturition in urethane anaesthetized female rats, by examining the effects of intravenous administration of various 5-HT_2 receptor agonists and antagonists on bladder and urethral responses evoked by saline infusion into the bladder. The effects of these agonists and antagonists on cardiovascular regulation were also investigated.

3.2 Results

Baseline values for all variables are shown in Tables 3.4-3.8.

3.2.1 Vehicle Control

Administration of 5-HT_2 receptor antagonist vehicle (0.9% wv^{-1} saline or 100% DMSO; i.v; n = 5) followed 5 minutes later by the 5-HT_2 receptor agonist vehicle (0.9% wv^{-1} saline; i.v; n = 5) evoked no significant changes in baseline EUS-EMG signal, urethral pressure and bladder distension caused by infusion of saline at a rate of 0.1 ml min^{-1} (micturition reflex). Baseline MAP and HR were also unaffected.

3.2.2 5-HT_2 receptor agonists i.v.

3.2.2.1 WAY 161503 (5-HT_{2C})

Traces showing the effects of WAY 161503 (300 μg kg^{-1}, i.v; n = 5) on changes in EUS-EMG signal, urethral pressure and the micturition reflex are shown in Figure 3.1a. Administration of WAY 161503 evoked EUS-EMG activity and significantly ($P < 0.05$) increased the EUS-EMG signal by 179 ± 17%. This was associated with a significant increase in urethral pressure reaching a maximum of 13 ± 2%. However, when compared to vehicle control, this increase was found to be significant only at one time point, 2 min (Figure 3.1b). The increase observed on EUS-EMG activity was found to be significant between 2 and 3 min. The onset of appearance of EUS-EMG activity was 28 ± 11s. This evoked increase in EUS-EMG activity was observed to be ongoing up to 10 min, at

which point the bladder was thereafter emptied and EUS-EMG activity ceased. In some experiments however, EUS-EMG activity continued over the period of evoking the micturition reflex.

WAY 161503 attenuated the micturition reflex causing a significant increase in volume threshold (55 ± 13%), pressure threshold (25 ± 11%) and residual volume (121 ± 35%; Figure 3.1c). However, WAY 161503 had no significant effect on the reflex-evoked urethral relaxation (data not illustrated). The mean area of integrated EUS-EMG burst that accompanied the bladder contraction tested in the presence of WAY 161503 was also unaffected. This was found to be the case with all the other experiments carried out throughout this study (except i.v. mCPP; see below), thus the reflex-evoked urethral relaxation and mean area of integrated EUS-EMG burst in the presence of compounds were not analysed any further.

WAY 161503 significantly increased MAP by 21 ± 1 mmHg, whereas HR was unaffected.

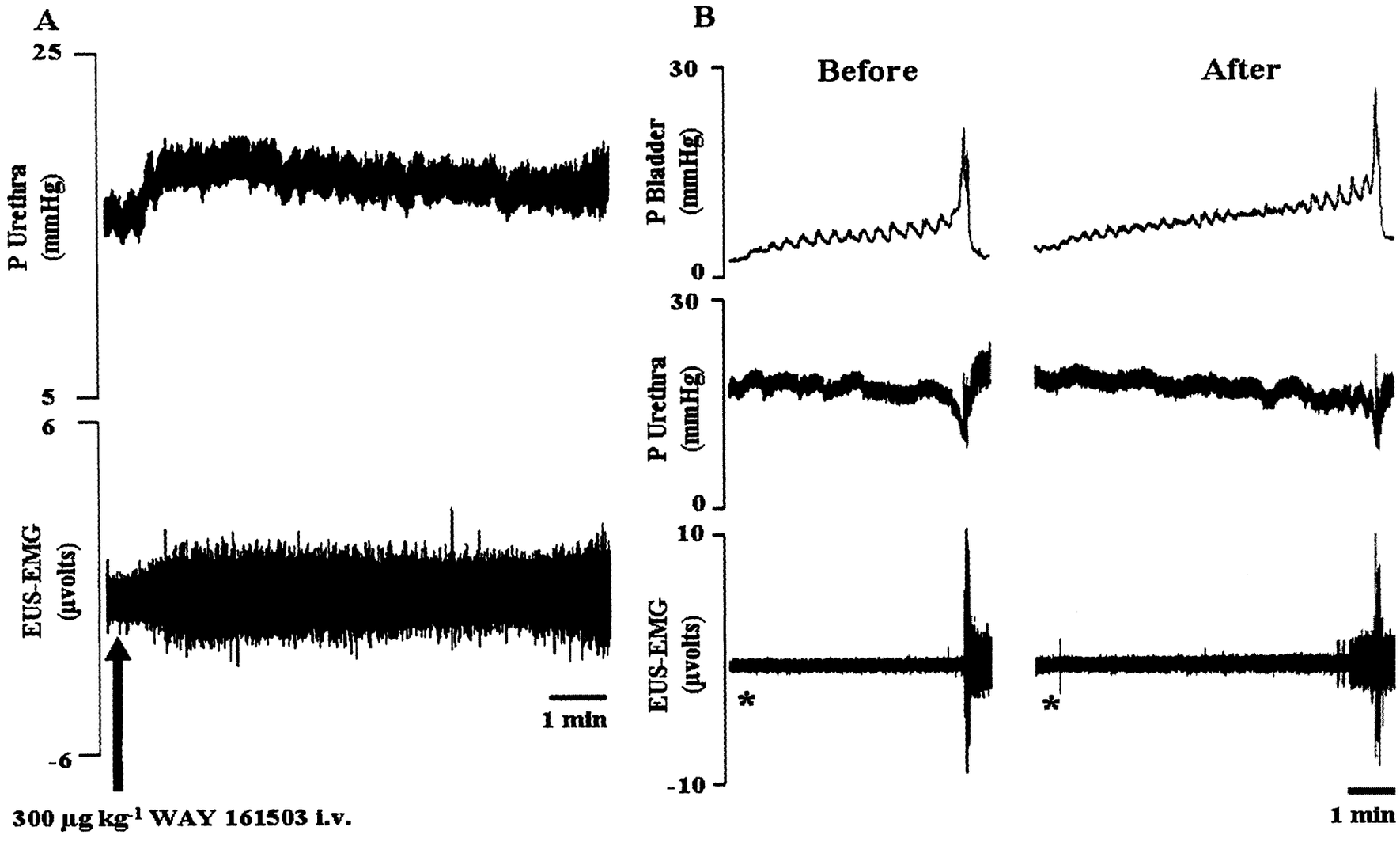

Figure 3.1a Traces showing the effects of WAY 161503 (300 µg kg^{-1}, i.v.) on **A** baseline urethral pressure and EUS-EMG activity and **B** bladder and urethral pressures and raw urethral striated muscle. * denotes onset of saline infusion into the bladder.

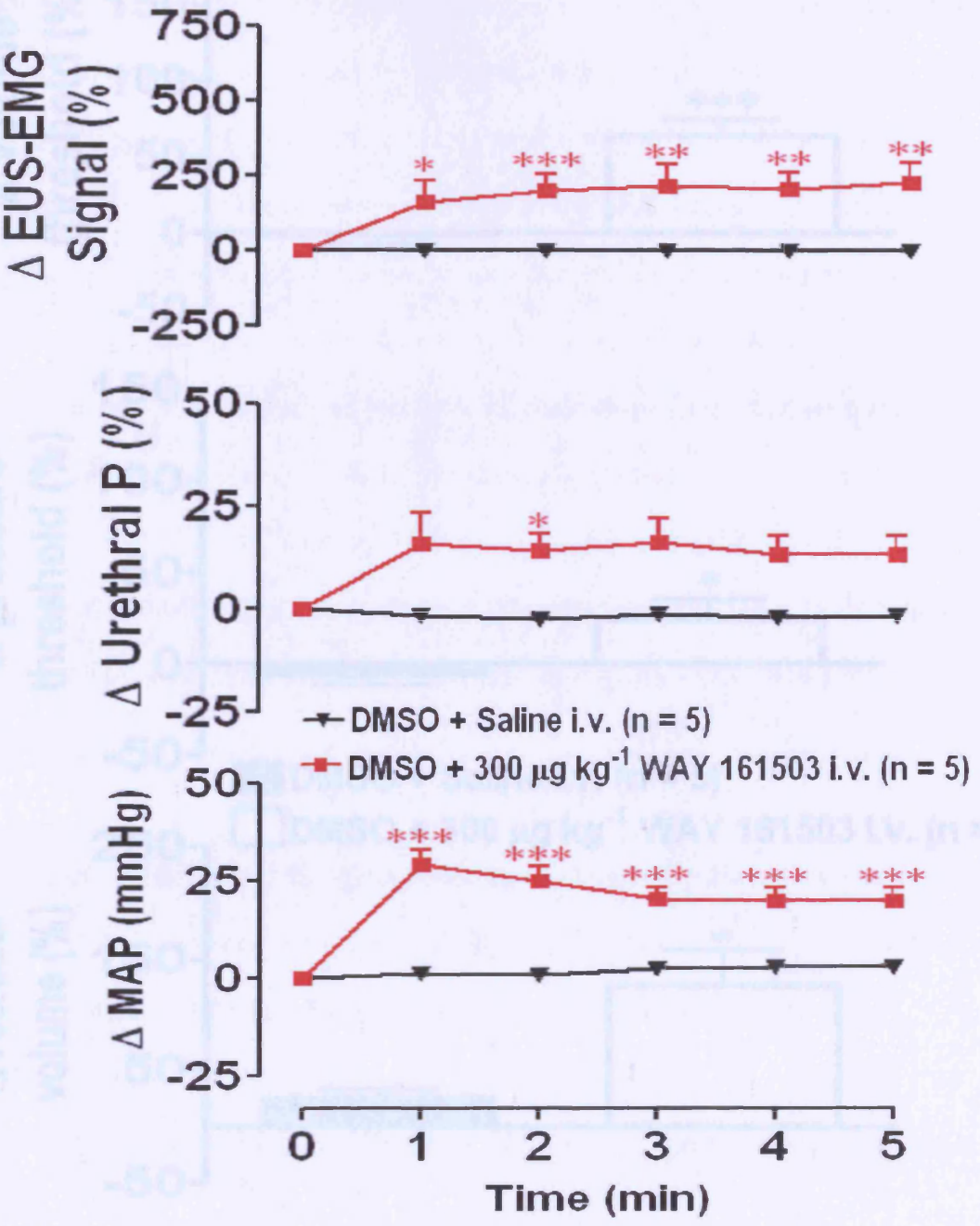

Figure 3.1b Urethane anaesthetised female rats: a comparison of the effects of vehicle (DMSO + saline) and WAY 161503 on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (UP) and mean arterial blood pressure (MAP). Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by WAY 161503 were compared with DMSO + saline control using two-way analysis of variance followed by the least significant difference test. * $P< 0.05$, *** $P<0.001$.

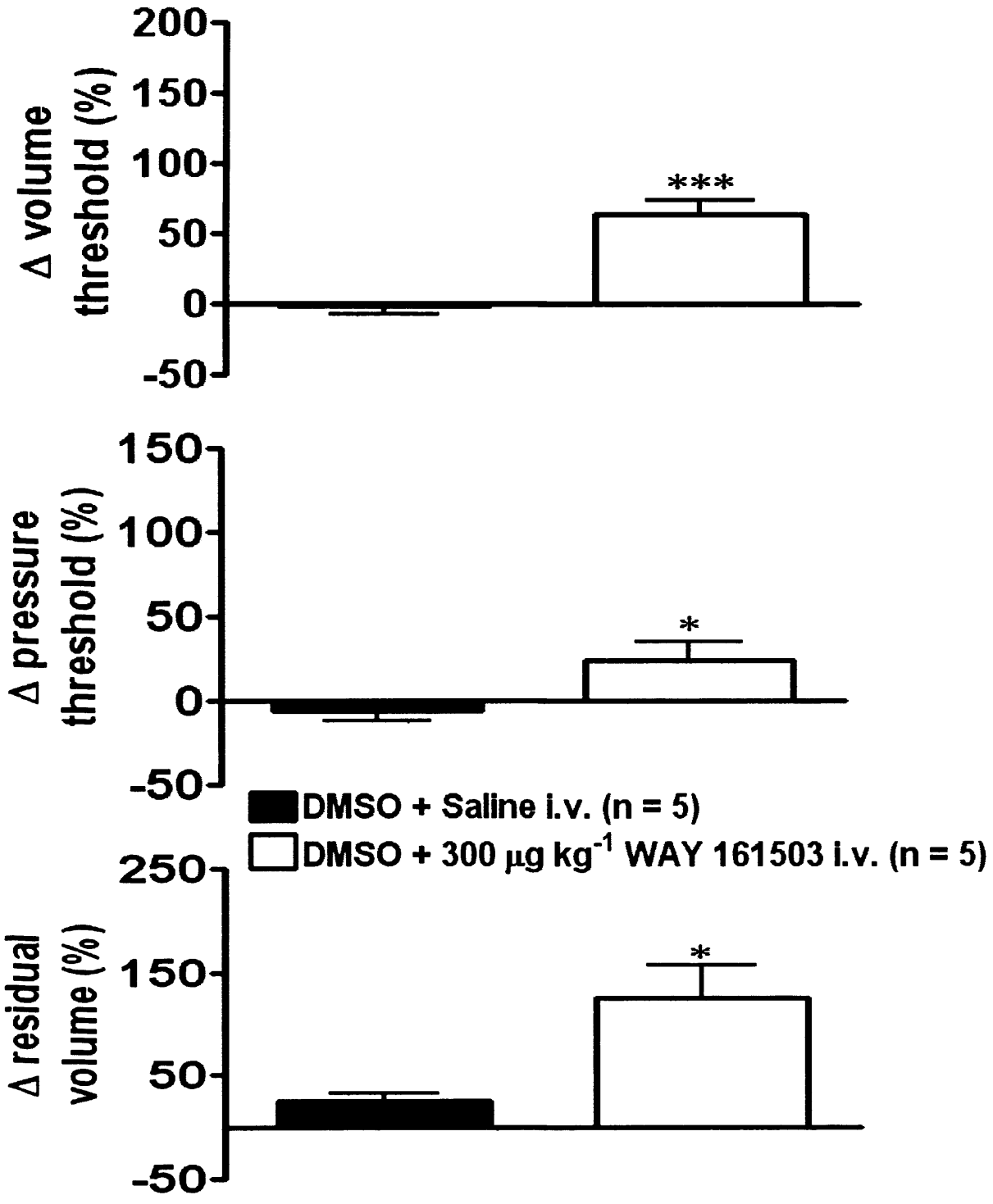

Figure 3.1c Urethane anaesthetised female rats: a comparison of the effects of vehicle (DMSO + saline) and WAY 161503 on percentage changes (Δ) in volume threshold, pressure threshold and residual volume. Each bar represents mean value and vertical bars show the s.e.mean. Changes caused by WAY 161503 were compared with DMSO + saline control using Student's unpaired t test. * $P< 0.05$, *** $P<0.001$.

3.2.2.2 Ro 60-0175 (5-HT_{2C})

Traces showing the effects of Ro 60-0175 (300 μg kg^{-1}, i.v; n = 5) on changes in EUS-EMG signal, urethral pressure and the micturition reflex are shown in Figure 3.2a. Ro 60-0175 (n = 5) evoked EUS-EMG activity and significantly increased the EUS-EMG signal by 154 ± 15%. This was associated with a significant increase in urethral pressure of 23 ± 2% (Figure 3.2b) which was maintained and thus found to be significant at all time points for the duration of the experiment. The onset of appearance of EUS-EMG activity was 36 ± 13s and this was paralleled by the increase in urethral pressure. Similar to WAY 161503 (see above), this evoked increase in EUS-EMG activity was observed to be ongoing up to 10 min. When the bladder was emptied to begin testing the micturition reflex, the EUS-EMG firing usually stopped. However, just like WAY 161503, in some experiments EUS-EMG activity was observed to be ongoing whilst testing the micturition reflex, see Figure 3.2a.

Ro 60-0175 also attenuated the micturition reflex causing a significant increase in volume threshold (71 ± 15%) and pressure threshold (90 ± 33%; Figure 3.2c), and although residual volume was increased, it was found not to be significant overall.

Ro 60-0175 significantly increased MAP by 14 ± 1 mmHg and decreased HR by -13 ± 2 beats/min.

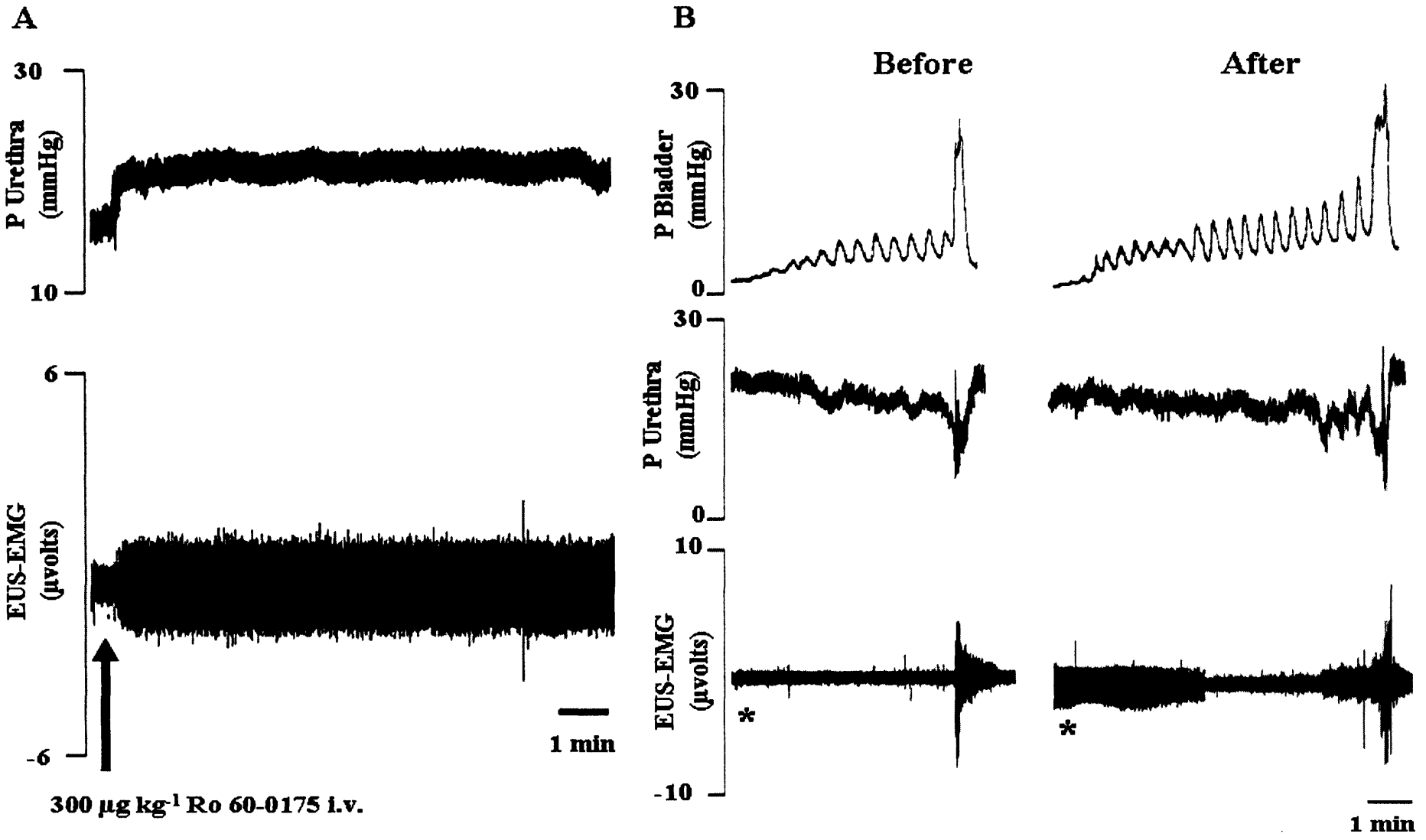

Figure 3.2a Trace showing the effects of Ro 60-0175 (300 µg kg^{-1}, i.v.) on **A** baseline urethral pressure and EUS-EMG activity and **B** bladder a urethral pressures and raw urethral striated muscle. * denotes onset of saline infusion into the bladder.

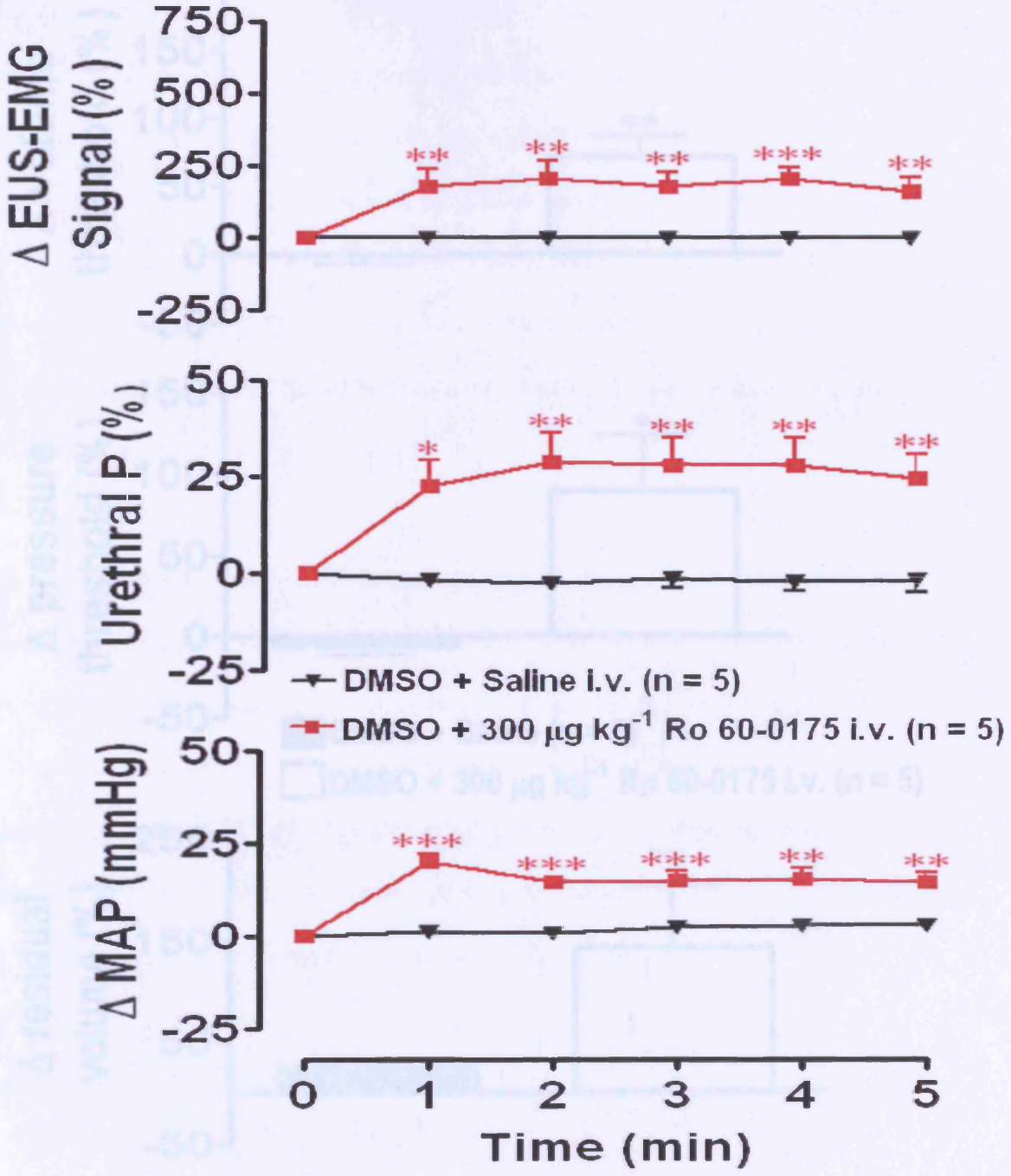

Figure 3.2b Urethane anaesthetised female rats: a comparison of the effects of vehicle (DMSO + saline) and Ro 60-0175 on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (UP) and mean arterial blood pressure (MAP). Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by Ro 60-0175 were compared with DMSO + saline control using two-way analysis of variance followed by the least significant difference test. * P< 0.05, ** P <0.01, *** P<0.001.

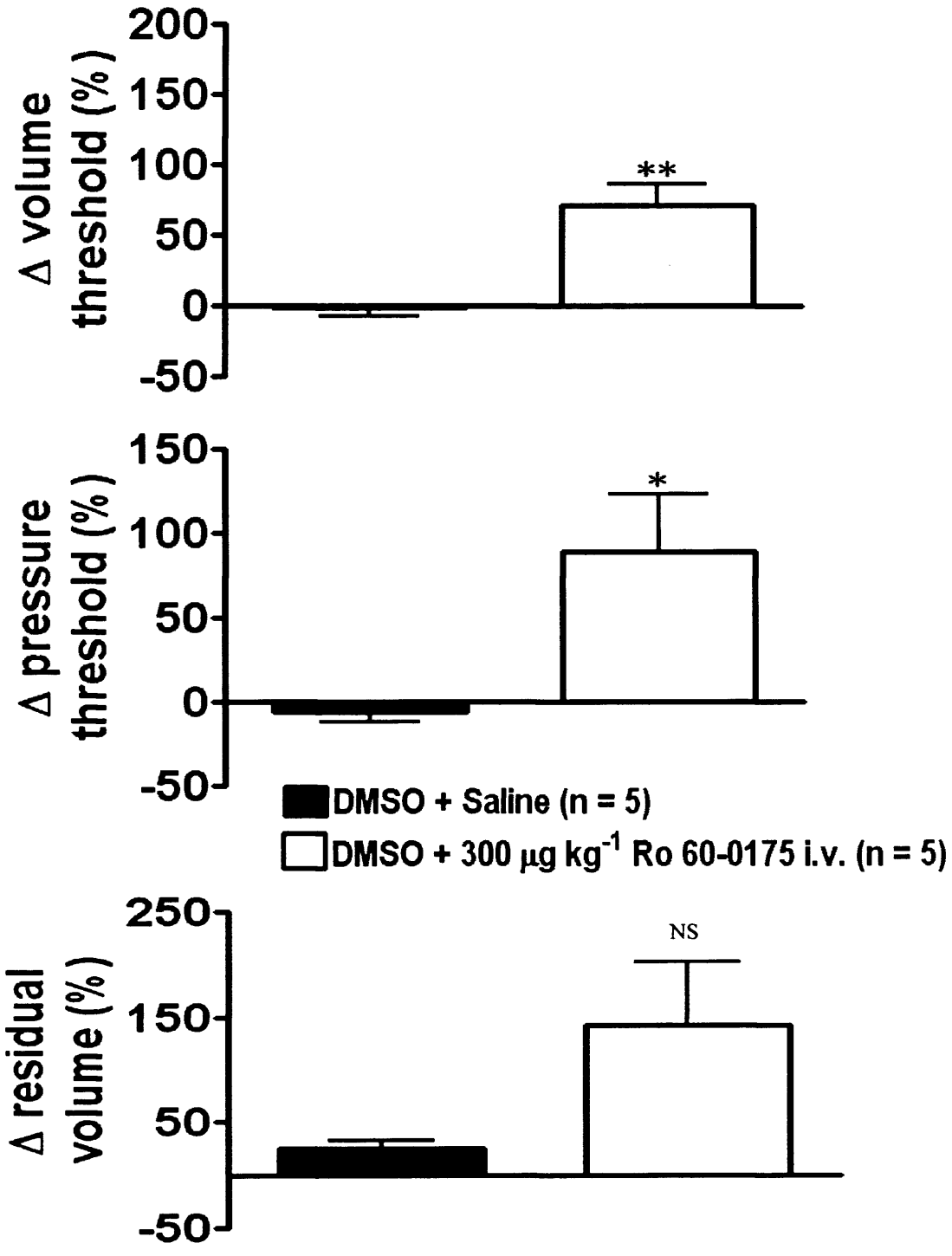

Figure 3.2c Urethane anaesthetised female rats: a comparison of the effects of vehicle (DMSO + saline) and Ro 60-0175 on percentage changes (Δ) in volume threshold, pressure threshold and residual volume. Each bar represents mean value and vertical bars show the s.e.mean. Changes caused by Ro 60-0175 were compared with DMSO + saline control using Student's unpaired t test. * $P< 0.05$, ** $P<0.01$, NS non-significant.

3.2.2.3 mCPP ($5\text{-}HT_{2C}$)

Traces showing the effects of mCPP (300 μg kg^{-1}, i.v; n = 5) on changes in EUS-EMG signal, urethral pressure and the micturition reflex are shown in Figure 3.3a. Saline and mCPP (n = 5) evoked EUS-EMG activity and significantly increased the EMG signal by 166 ± 17%. This was associated with a significant increase in urethral pressure of 24 ± 3% (Figure 3.3b) which although the increase observed on urethral pressure was parallel with the appearance of EUS-EMG activity; it did not reach significance until after 5 min. The onset of appearance of EUS-EMG activity was 20 ± 5s and the duration of EUS-EMG firing was between 5-8 min.

mCPP completely blocked the micturition reflex causing a continuous rise in bladder pressure resulting after 5 min in the appearance of a few drops of saline leaking from the urethral orifice. Such responses were taken to indicate that the micturition reflex had been abolished (see Figure 3.3a). As there was no obvious bladder contraction following administration of mCPP, both the reflex-evoked urethral relaxation and mean area of integrated EUS-EMG burst that accompanied the bladder contraction were found to be markedly reduced or not present at all, as seen in Figure 3.3a.

Baseline MAP and HR were unaffected by mCPP.

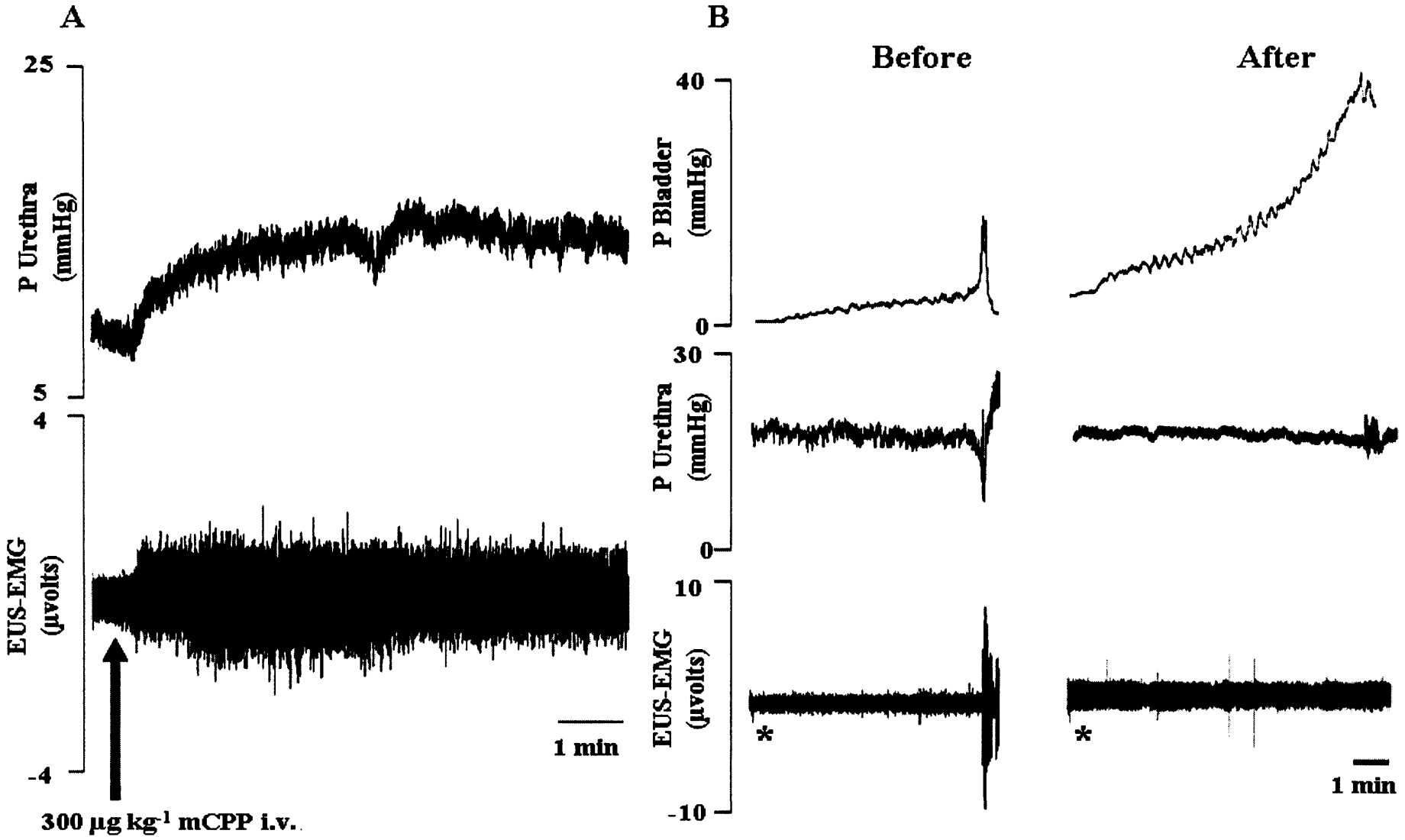

Figure 3.3a Traces showing the effects of mCPP (300 µg kg^{-1}, i.v.) on **A** baseline urethral pressure and EUS-EMG activity and **B** bladder and urethral pressures and raw urethral striated muscle. * denotes onset of saline infusion into the bladder.

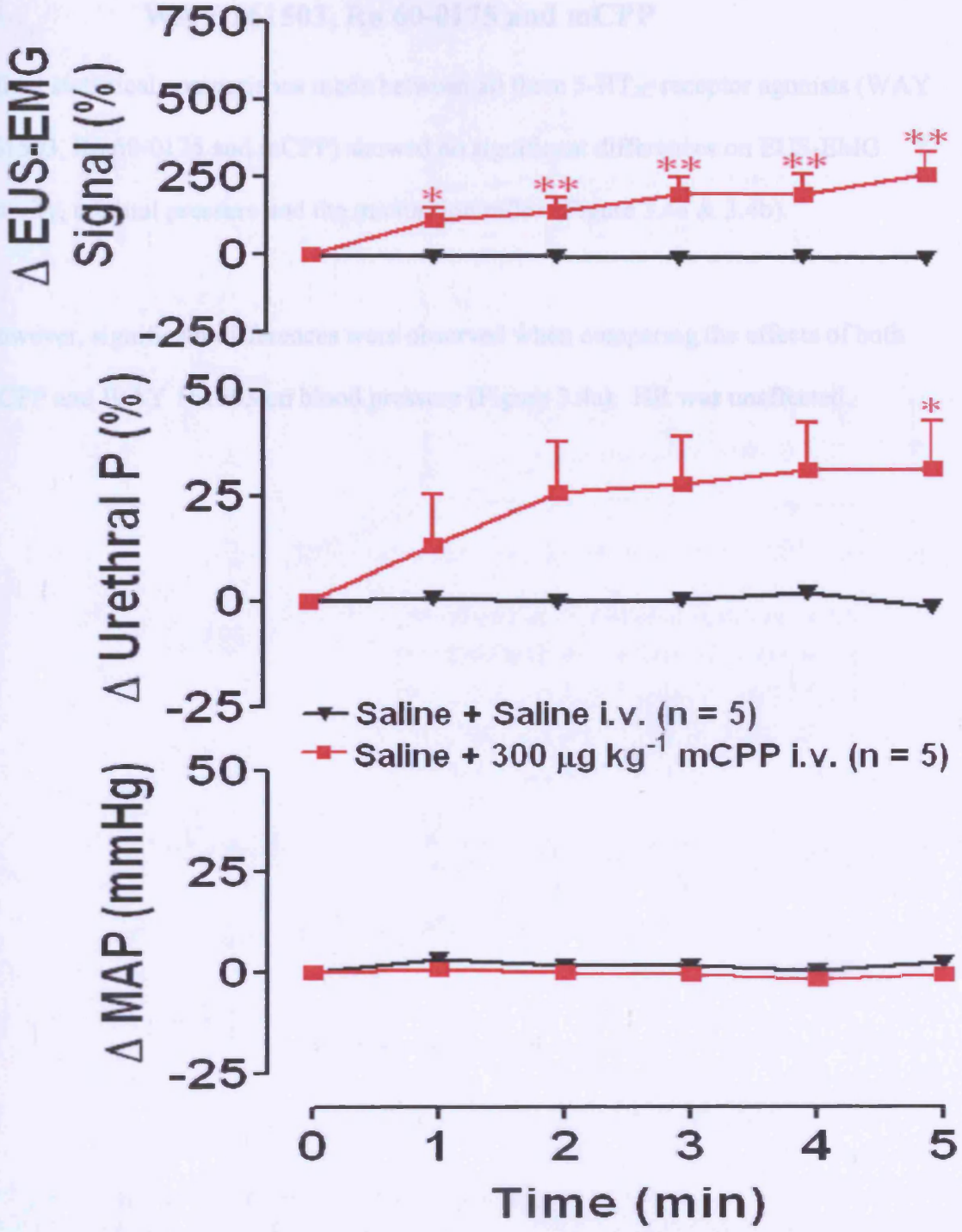

Figure 3.3b Urethane anaesthetised female rats: a comparison of the effects of vehicle (saline + saline) and mCPP on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (UP) and mean arterial blood pressure (MAP). Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by mCPP were compared with saline + saline control using two-way analysis of variance followed by the least significant difference test. * $P< 0.05$, ** $P <0.01$.

3.2.2.4 Comparisons made between 5-HT_{2C} receptor agonists; WAY 161503, Ro 60-0175 and mCPP

Direct statistical comparisons made between all three 5-HT_{2C} receptor agonists (WAY 161503, Ro 60-0175 and mCPP) showed no significant differences on EUS-EMG activity, urethral pressure and the micturition reflex (Figure 3.4a & 3.4b).

However, significant differences were observed when comparing the effects of both mCPP and WAY 161503 on blood pressure (Figure 3.4a). HR was unaffected.

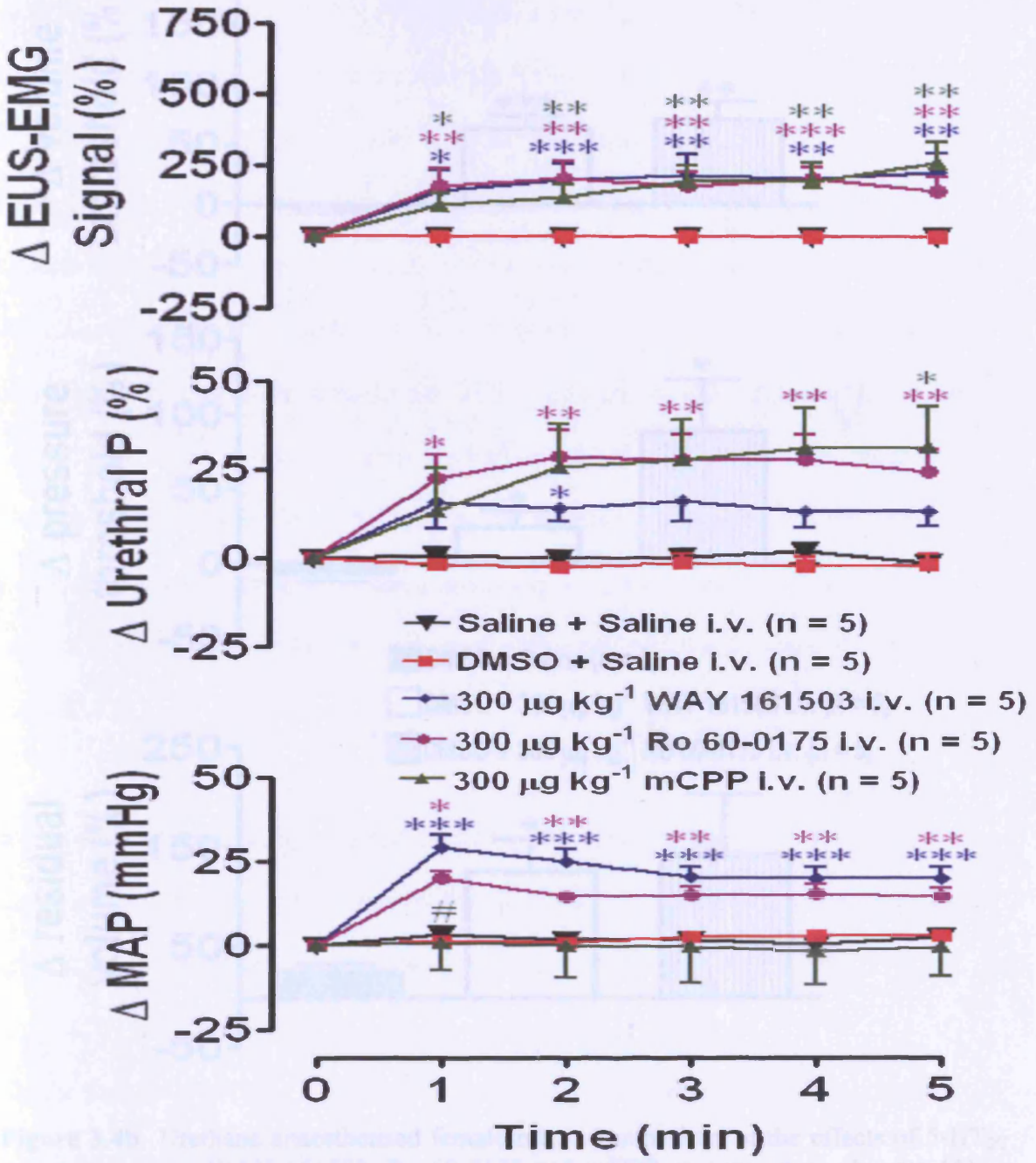

Figure 3.4a Urethane anaesthetised female rats: a comparison of the effects of 5-HT_{2C} receptor agonists WAY 161503, Ro 60-0175 and mCPP on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (UP) and mean arterial blood pressure (MAP). Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by WAY 161503, Ro 60-0175 and mCPP were compared with saline or DMSO + saline control using two-way analysis of variance followed by the least significant difference test. *, # $P<0.05$, ** $P<0.01$, *** $P<0.001$. (*), compared to saline or DMSO + saline, (#), compared to DMSO + WAY 161503.

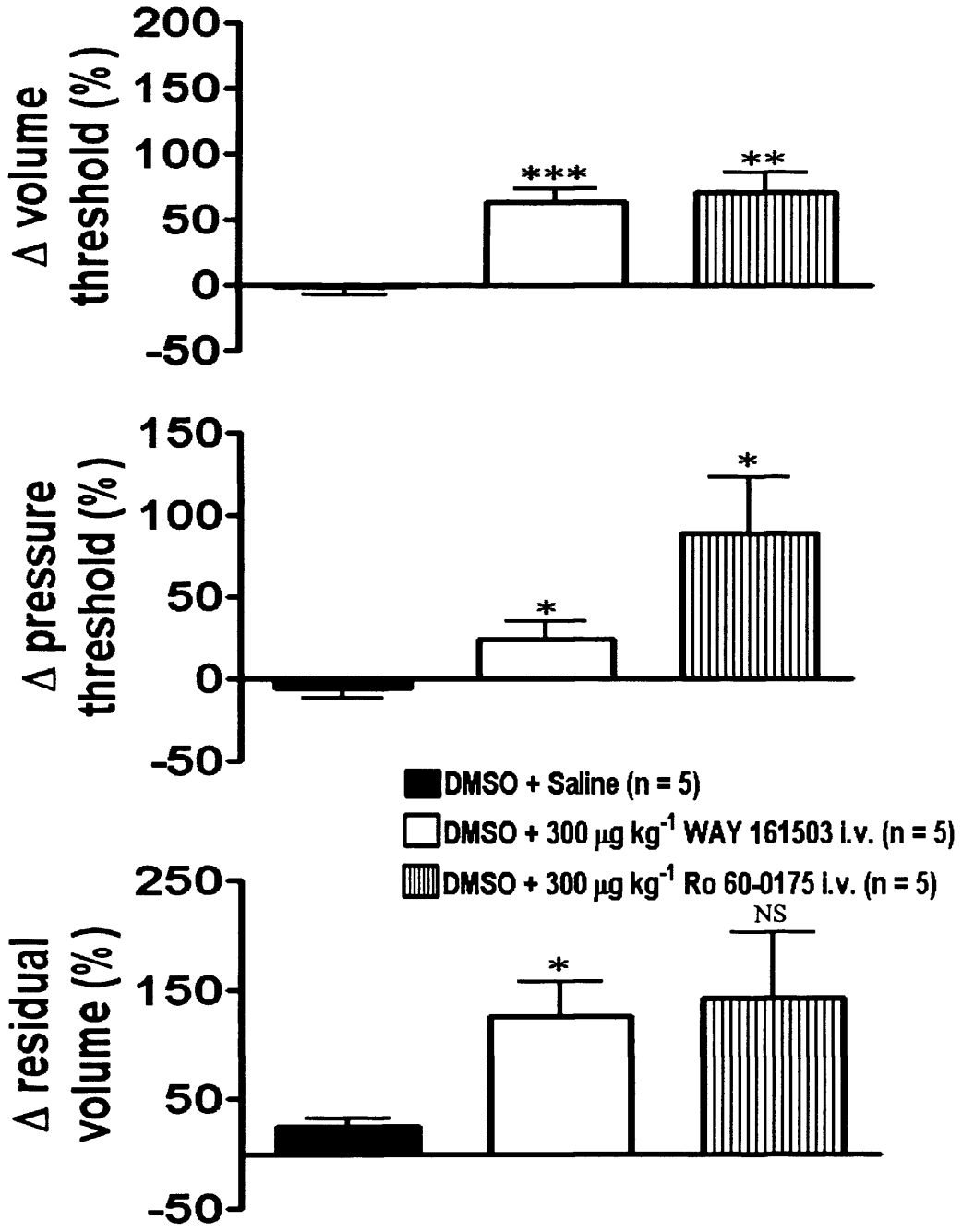

Figure 3.4b Urethane anaesthetised female rats: a comparison of the effects of 5-HT_{2C} receptor agonists WAY 161503, Ro 60-0175 and mCPP on percentage changes (Δ) in volume threshold, pressure threshold and residual volume. Each bar represents mean value and vertical bars show the s.e.mean. Changes caused by WAY 161503, Ro 60-0175 and mCPP were compared with DMSO + saline control using Student's unpaired t test. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, NS non-significant.
mCPP was not included as part of the reflex data as the reflex was completely abolished with no obvious bladder contraction observed hence unable to measure any of the bladder variables.

3.2.2.5 DOI (5-HT_{2A})

Traces showing the effects of DOI (100 μg kg^{-1}) on changes in EUS-EMG signal, urethral pressure and the micturition reflex are shown in Figure 3.5a. DOI (30, 50 and 100μg kg^{-1}, n = 3/5) in separate experiments increased baseline EUS-EMG signal activity by 139 ± 27%, 224 ± 36% and 198 ± 15% respectively (Figure 3.5b). However, these increases were found not to be dose related on the EUS-EMG signal. The 3 doses of DOI had no significant effect on urethral pressure (Figure 3.5b). Onset of appearance of EUS-EMG activity following administration of DOI (30, 50 and 100 μg kg^{-1}) was 48 ± 9s, 46 ± 6s and 17 ± 3s respectively. This evoked increase in EUS-EMG activity was observed to be ongoing up to 10 min. When the bladder was emptied to test the micturition reflex, EUS-EMG firing usually stopped, but again in some experiments EUS-EMG was observed to be ongoing whilst testing the micturition reflex in the presence of all three doses of DOI.

On the micturition reflex, both 30 and 100μg kg^{-1} DOI were found to be excitatory, causing a significant decrease in volume threshold (-27 ± 5% and -35 ± 7% respectively; Figure 3.5c), but again showing no dose relationship.

Only the high dose of DOI (100 μg kg^{-1}) significantly increased MAP by 21 ± 1 mmHg (Figure 3.5b). Overall, DOI at all three doses had no effect on HR.

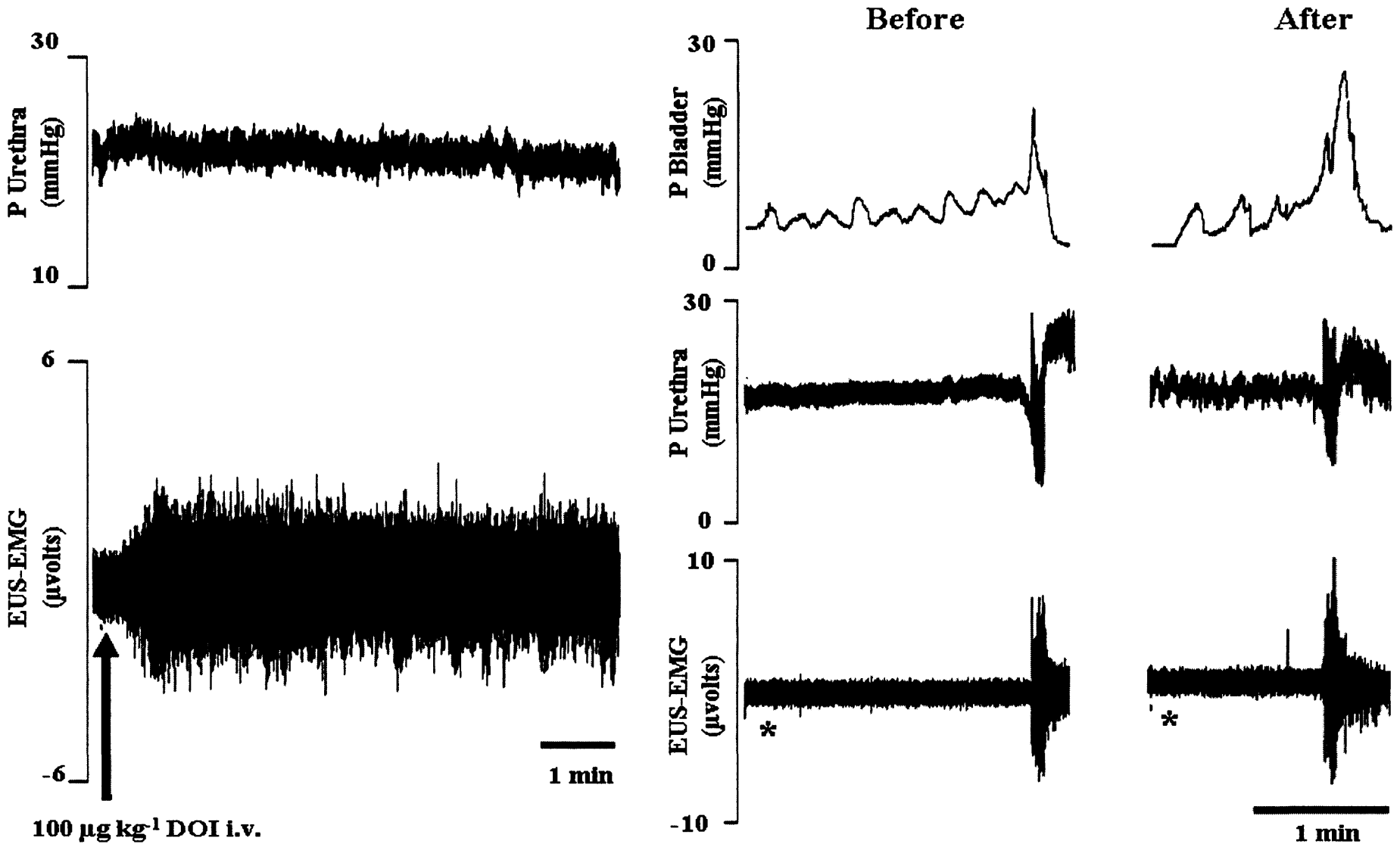

Figure 3.5a Trace showing the effects of DOI (100 µg kg^{-1}, i.v.) on **A** baseline urethral pressure and EUS-EMG activity and **B** bladder and urethral pressures and raw urethral striated muscle. * denotes onset of saline infusion into the bladder.

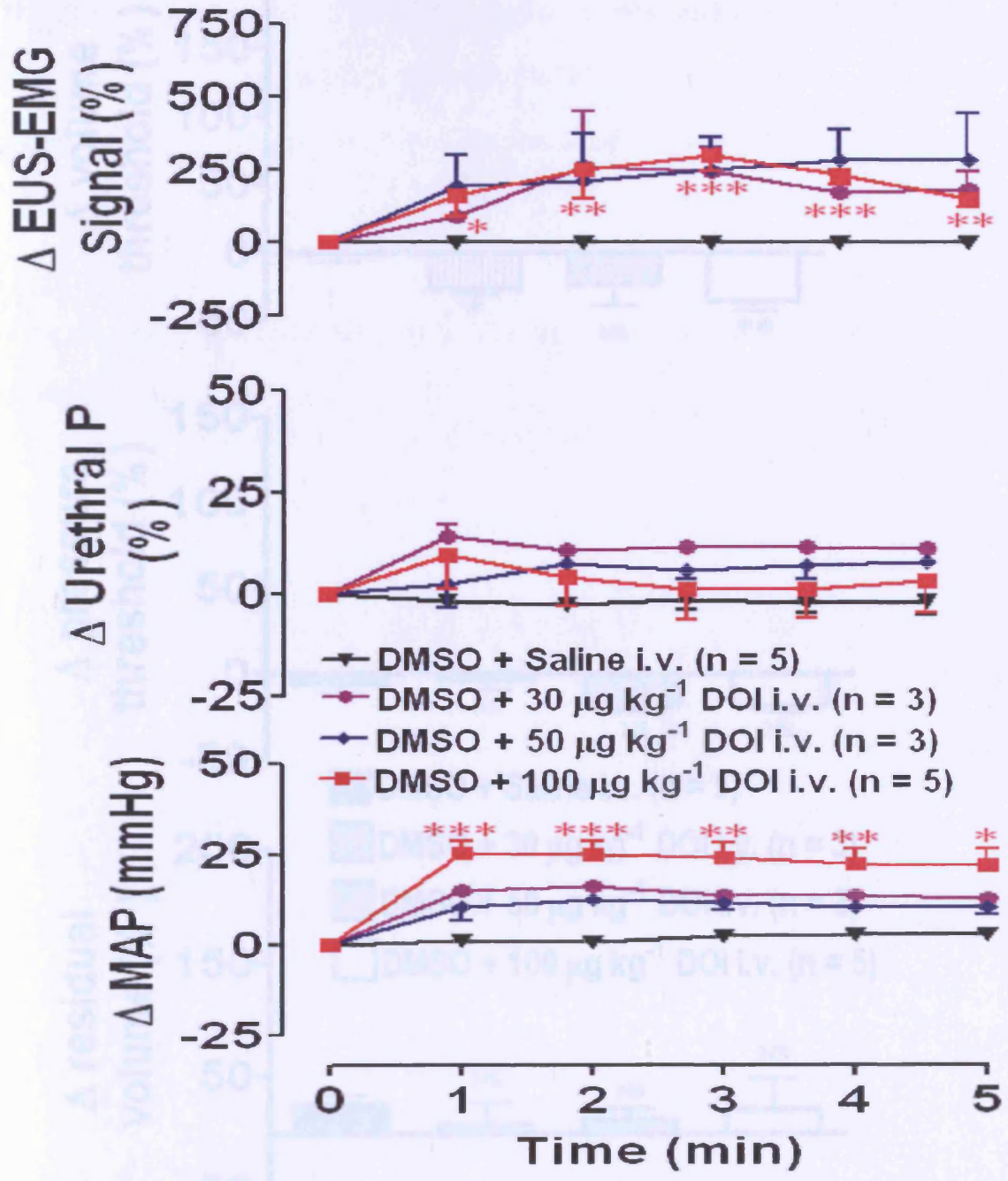

Figure 3.5b Urethane anaesthetised female rats: a comparison of the effects of vehicle (DMSO + saline) and DOI on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (UP) and mean arterial blood pressure (MAP). Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by DOI were compared with DMSO + saline control using two-way analysis of variance followed by the least significant difference test. * $P< 0.05$, ** $P <0.01$, *** $P<0.001$.

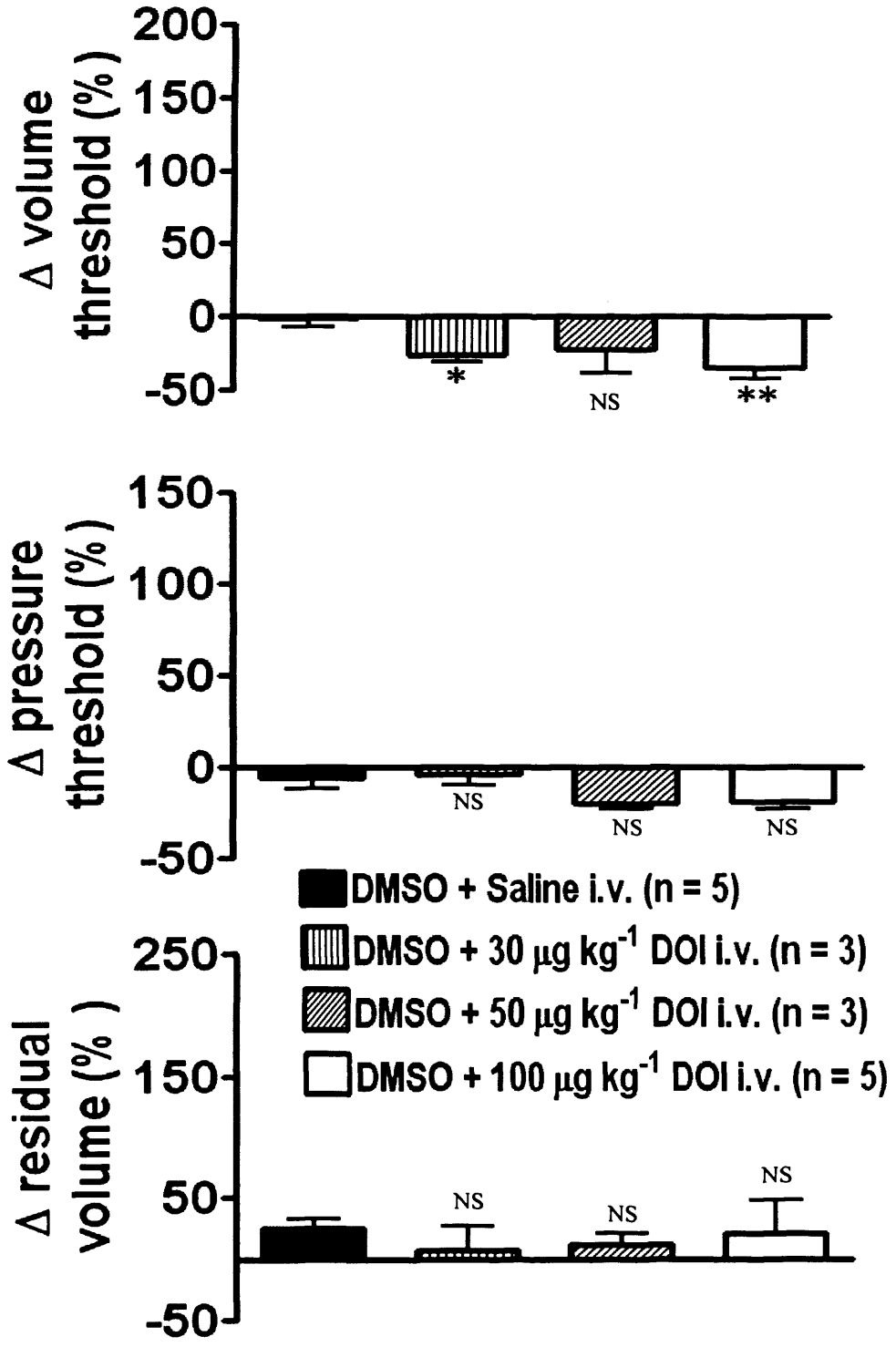

Figure 3.5c Urethane anaesthetised female rats: a comparison of the effects of vehicle (DMSO + saline) and DOI on percentage changes (Δ) in volume threshold, pressure threshold and residual volume. Each bar represents mean value and vertical bars show the s.e.mean. Changes caused by DOI were compared with DMSO + saline control using Student's unpaired t test. * $P< 0.05$, ** $P<0.01$, NS non-significant.

3.2.2.6 BW723C86 (5-HT_{2B})

BW723C86 (300 μg kg^{-1}, i.v; n = 5) had no significant effect on baseline EUS-EMG signal, MAP and HR (Figure 3.6a). Although BW723C86 appeared to cause an increase in urethral pressure between 4 and 5 min, this was found not to be significant overall (Figure 3.6a).

On the micturition reflex, BW723C86 significantly increased pressure threshold (30 ± 9%; Figure 3.6b)

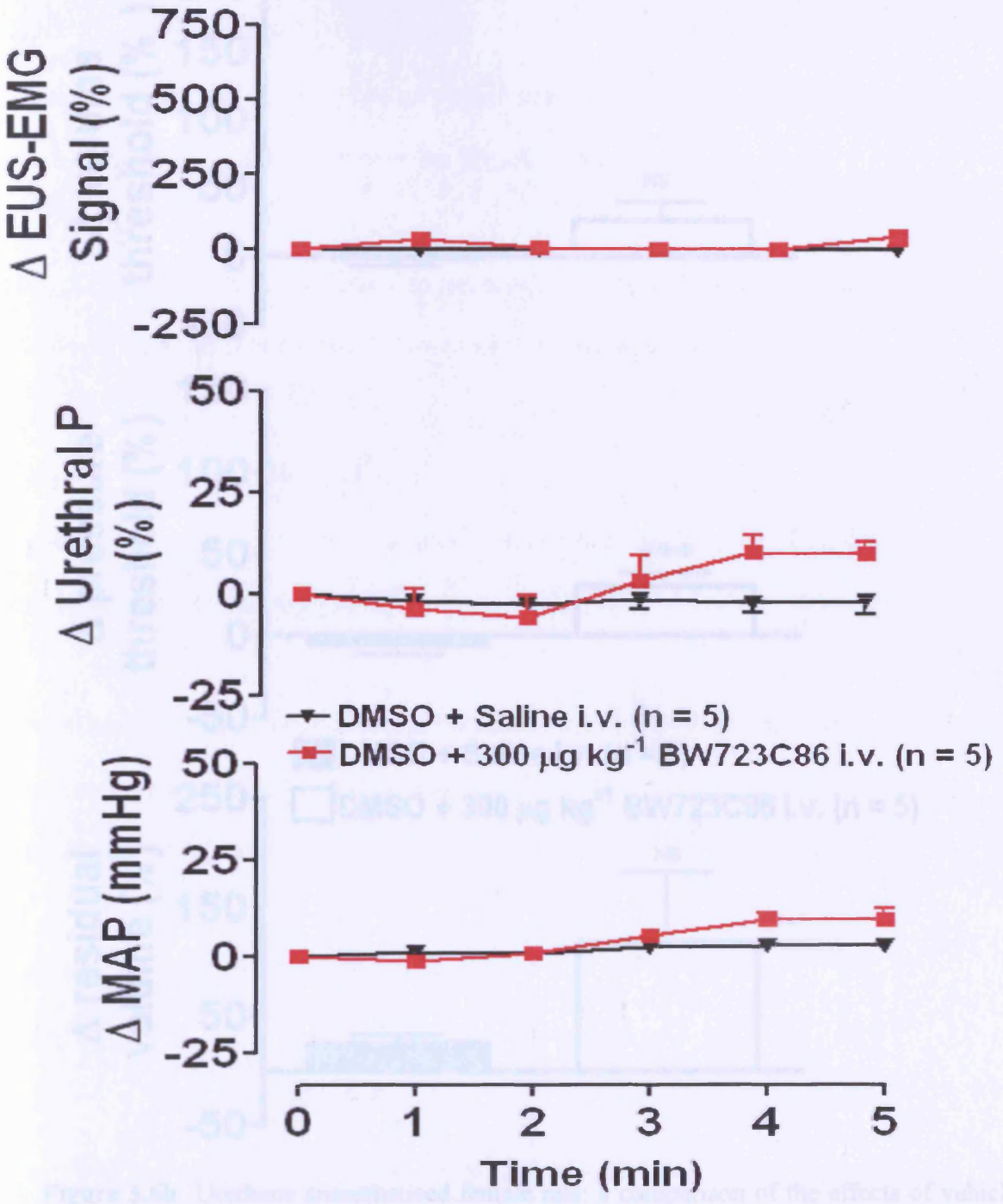

Figure 3.6a Urethane anaesthetised female rats: a comparison of the effects of vehicle (DMSO + saline) and BW723C86 on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (UP) and mean arterial blood pressure (MAP). Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by BW723C86 were compared with DMSO + saline control using two-way analysis of variance followed by the least significant difference test.

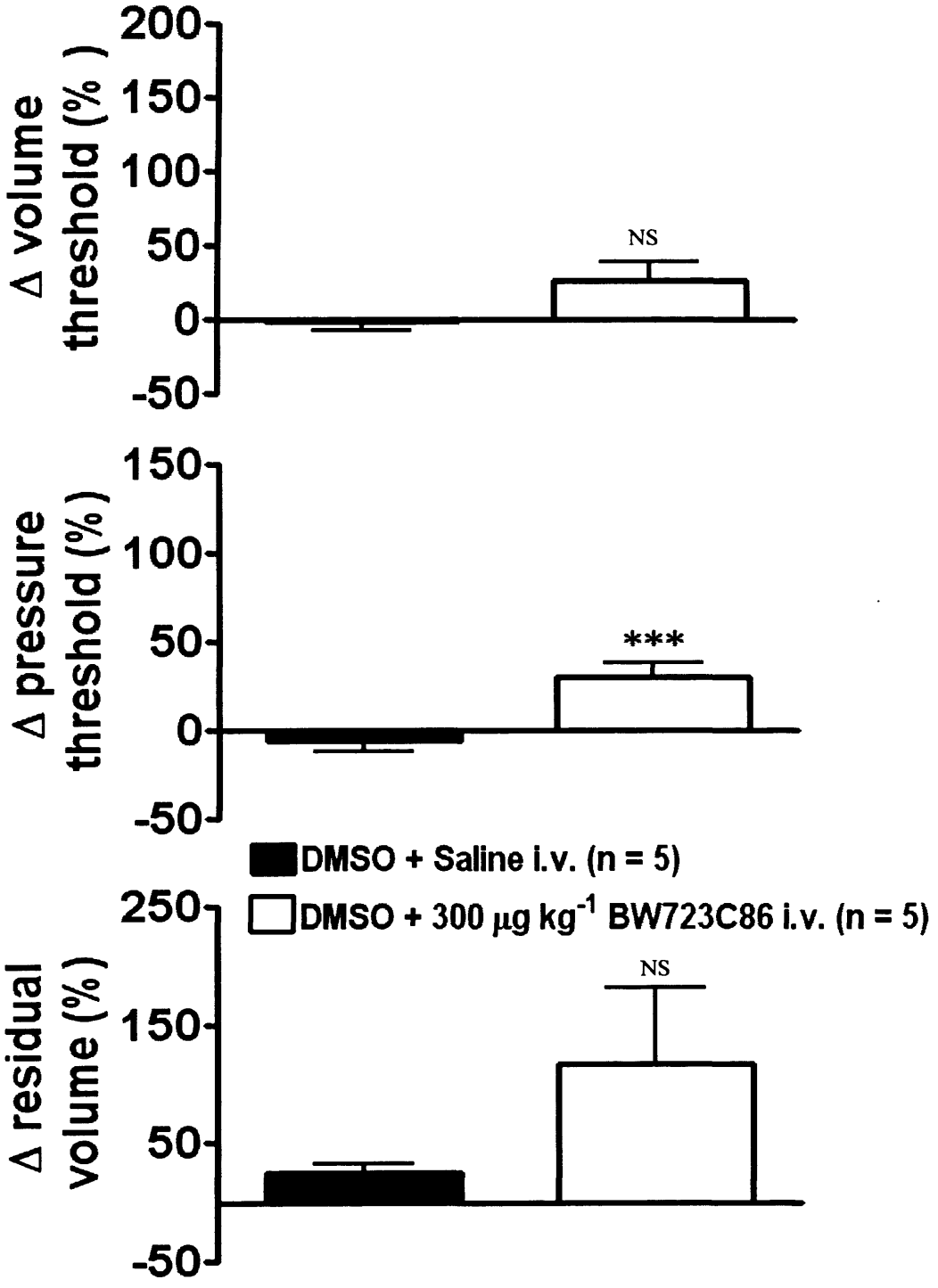

Figure 3.6b Urethane anaesthetised female rats: a comparison of the effects of vehicle (DMSO + saline) and BW723C86 on percentage changes (Δ) in volume threshold, pressure threshold and residual volume. Each bar represents mean value and vertical bars show the s.e.mean. Changes caused by BW723C86 were compared with DMSO + saline control using Student's unpaired t test. *** $P<0.001$, NS non-significant.

3.2.3 5-HT_2 receptor antagonists i.v.

3.2.3.1 Mianserin (5-$HT_{2A/2B/2C}$)

Mianserin (300 μg kg^{-1}, i.v; n = 5) had no significant effect on baseline EUS-EMG signal, urethral pressure and the micturition reflex (Figure 3.7a and 3.7b).

Although mianserin appeared to cause an increase in MAP (see Figure 3.7a), this was found not to be significant overall. Mianserin had no effect on HR.

3.2.3.2 Ketanserin (5-HT_{2A})

Ketanserin (100 μg kg^{-1}, i.v; n = 5) had no significant effect on baseline EUS-EMG signal and urethral pressure (Figure 3.8a).

On the micturition reflex, ketanserin was inhibitory, with a significant increase observed on volume threshold (27 ± 3%) and pressure threshold (11 ± 4%; Figure 3.8b), but had no effect on residual volume.

Ketanserin had no effect on either MAP or HR.

3.2.3.3 MDL 100907 (5-HT_{2A})

MDL 100907 (30 μg kg^{-1}, i.v; n = 5) and saline had no significant effect on baseline EUS-EMG signal and urethral pressure (Figure 3.9a).

On the micturition reflex, MDL 100907 was inhibitory, with a significant increase observed on volume threshold (65 ± 15%; Figure 3.9b) but not pressure threshold or residual volume.

MDL 100907 significantly increased MAP by 14 ± 1 mmHg (Figure 3.9a). HR was unaffected.

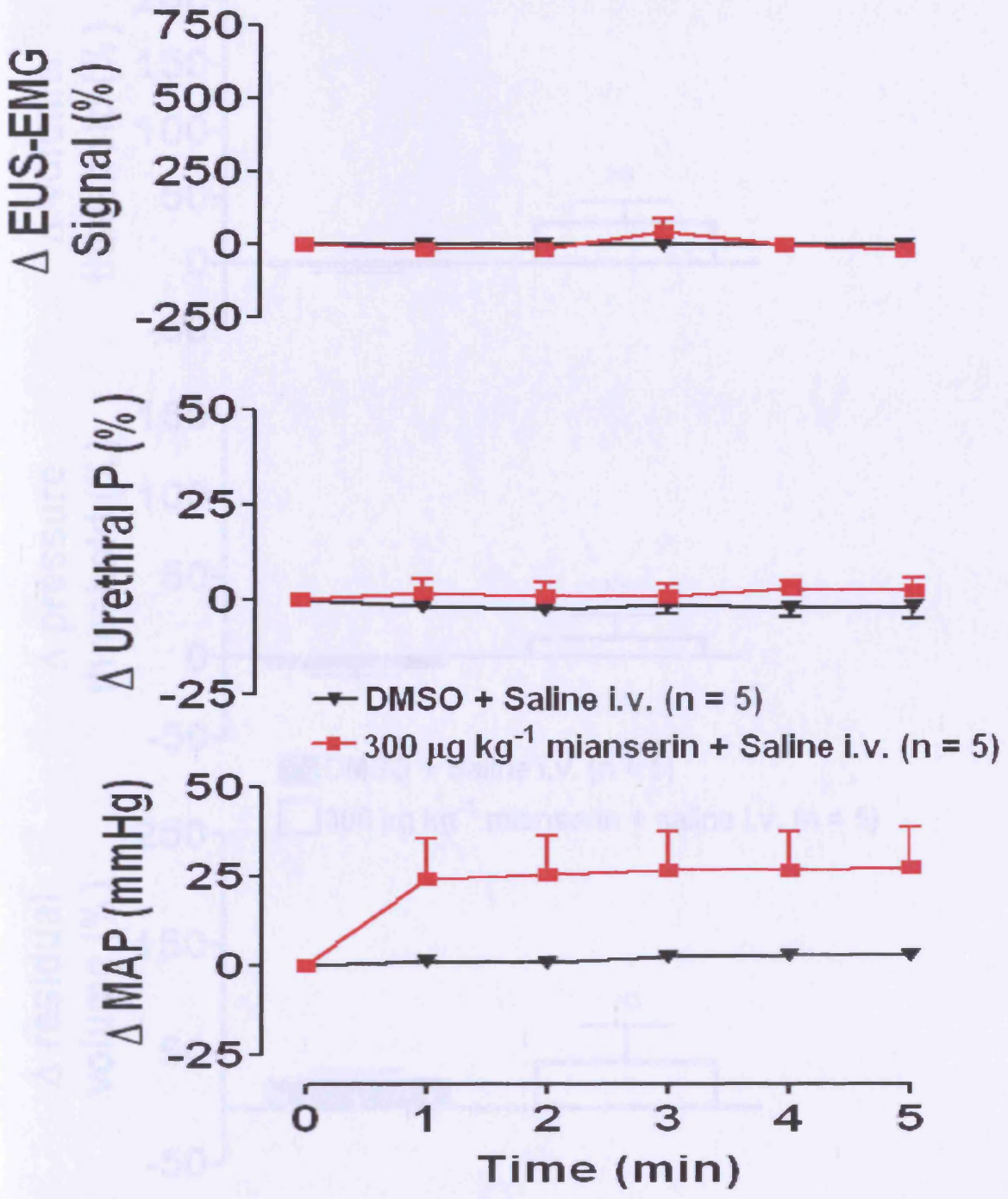

Figure 3.7a Urethane anaesthetised female rats: a comparison of the effects of mianserin and vehicle (saline) on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (UP) and mean arterial blood pressure (MAP). Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by mianserin and vehicle were compared with DMSO + saline control using two-way analysis of variance followed by the least significant difference test.

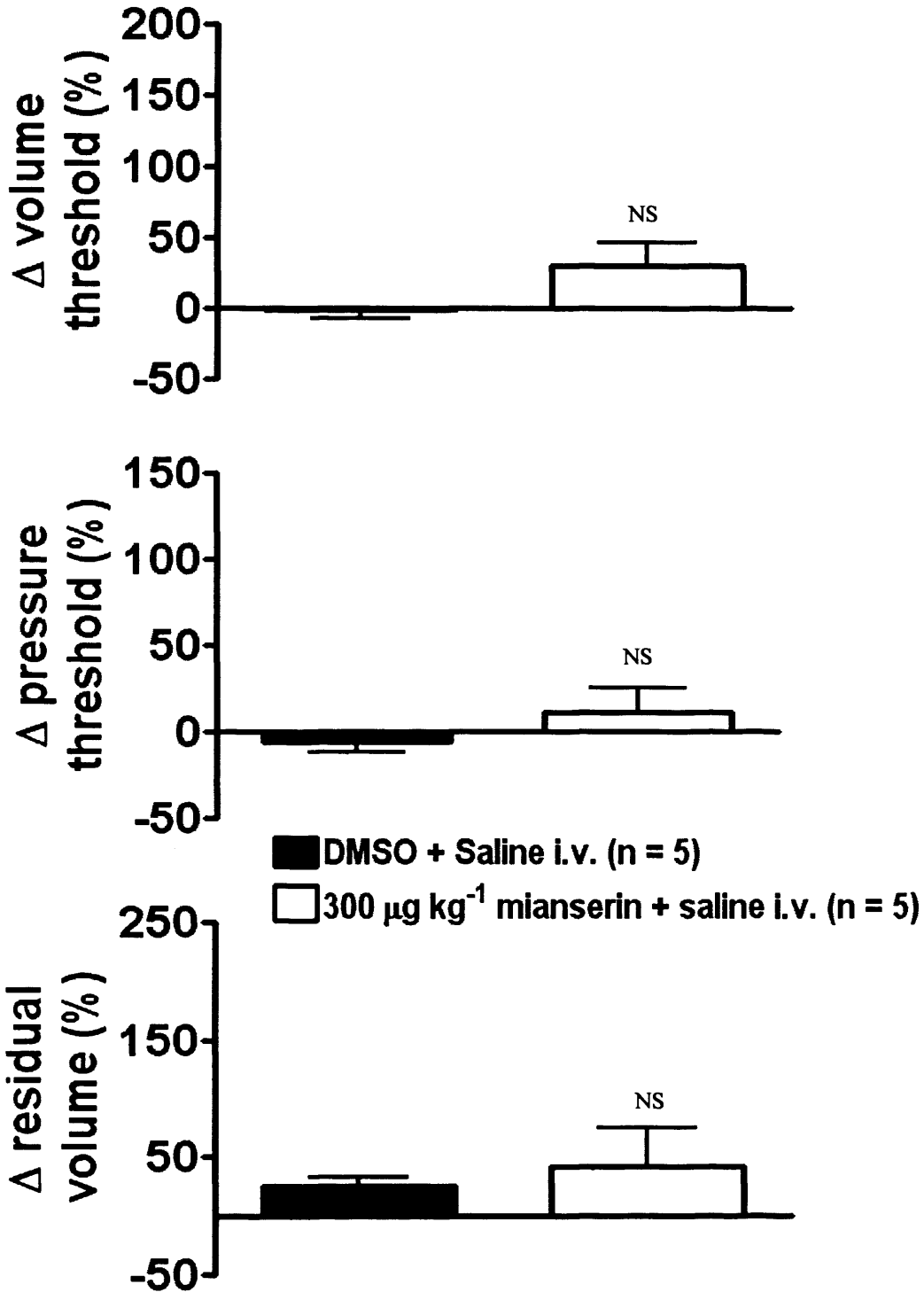

Figure 3.7b Urethane anaesthetised female rats: a comparison of the effects of mianserin and vehicle (saline) on percentage changes (Δ) in volume threshold, pressure threshold and residual volume. Each bar represents mean value and vertical bars show the s.e.mean. Changes caused by mianserin and saline were compared with DMSO + saline control using Student's unpaired t test. NS non-significant.

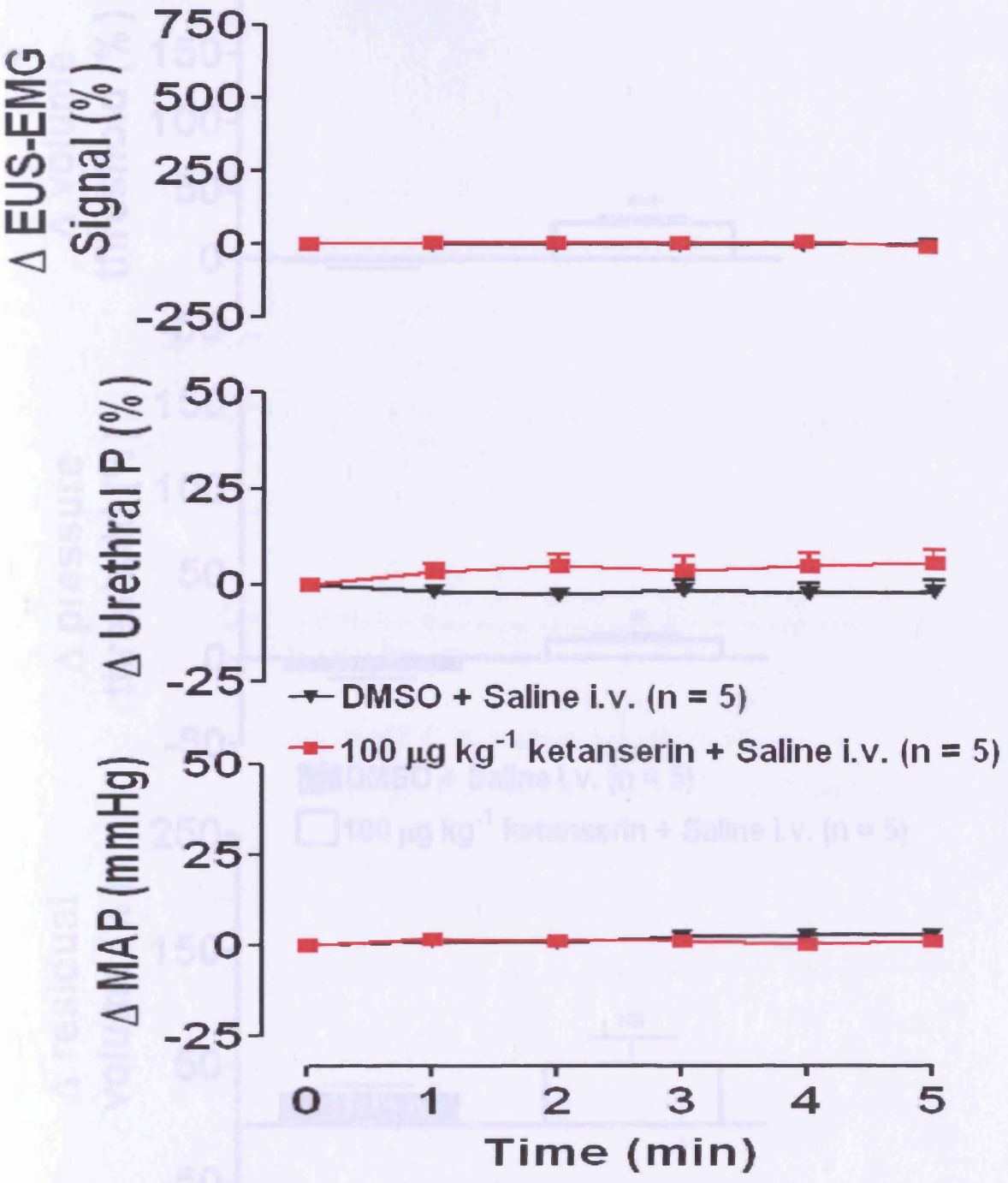

Figure 3.8a Urethane anaesthetised female rats: a comparison of the effects of ketanserin and vehicle (saline) on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (UP) and mean arterial blood pressure (MAP). Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by ketanserin and vehicle were compared with DMSO + saline control using two-way analysis of variance followed by the least significant difference test.

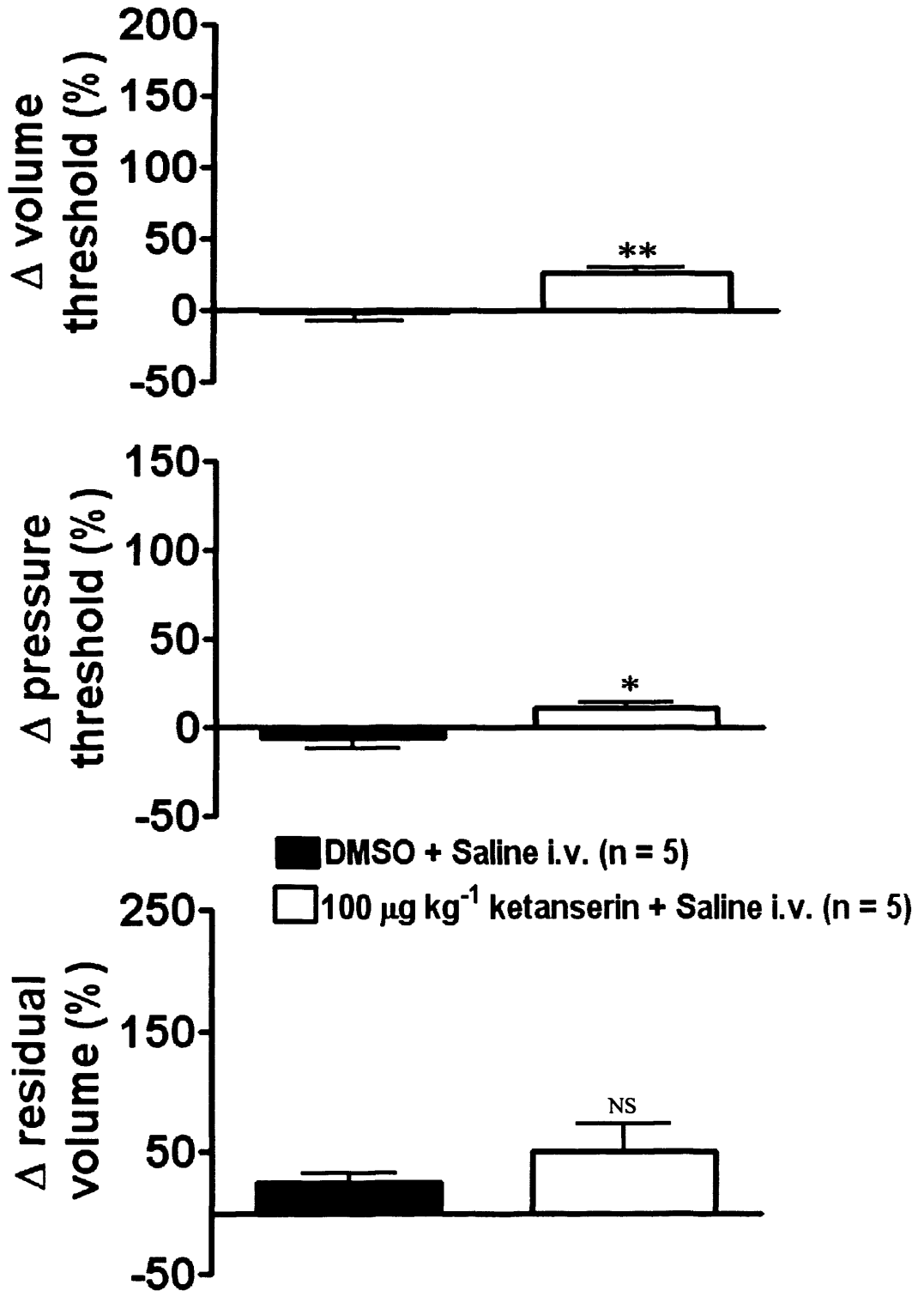

Figure 3.8b Urethane anaesthetised female rats: a comparison of the effects of ketanserin and vehicle (saline) on percentage changes (Δ) in volume threshold, pressure threshold and residual volume. Each bar represents mean value and vertical bars show the s.e.mean. Changes caused by ketanserin and saline were compared with DMSO + saline control using Student's unpaired t test. * $P<0.05$, ** $P<0.01$, NS non-significant.

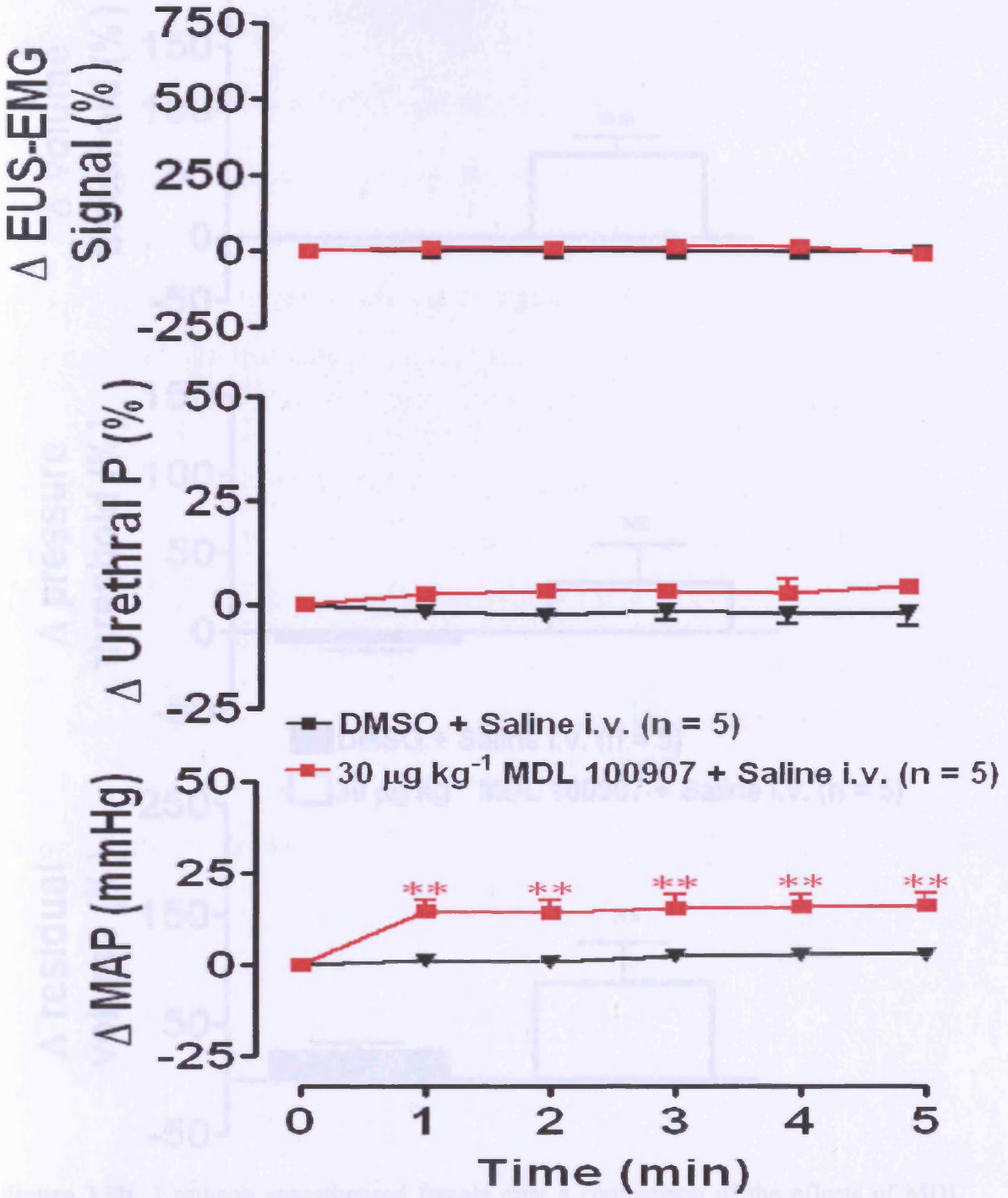

Figure 3.9a Urethane anaesthetised female rats: a comparison of the effects of MDL 100907 and vehicle (saline) on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (UP) and mean arterial blood pressure (MAP). Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by MDL 100907 and vehicle were compared with DMSO + saline control using two-way analysis of variance followed by the least significant difference test. ** $P<0.01$.

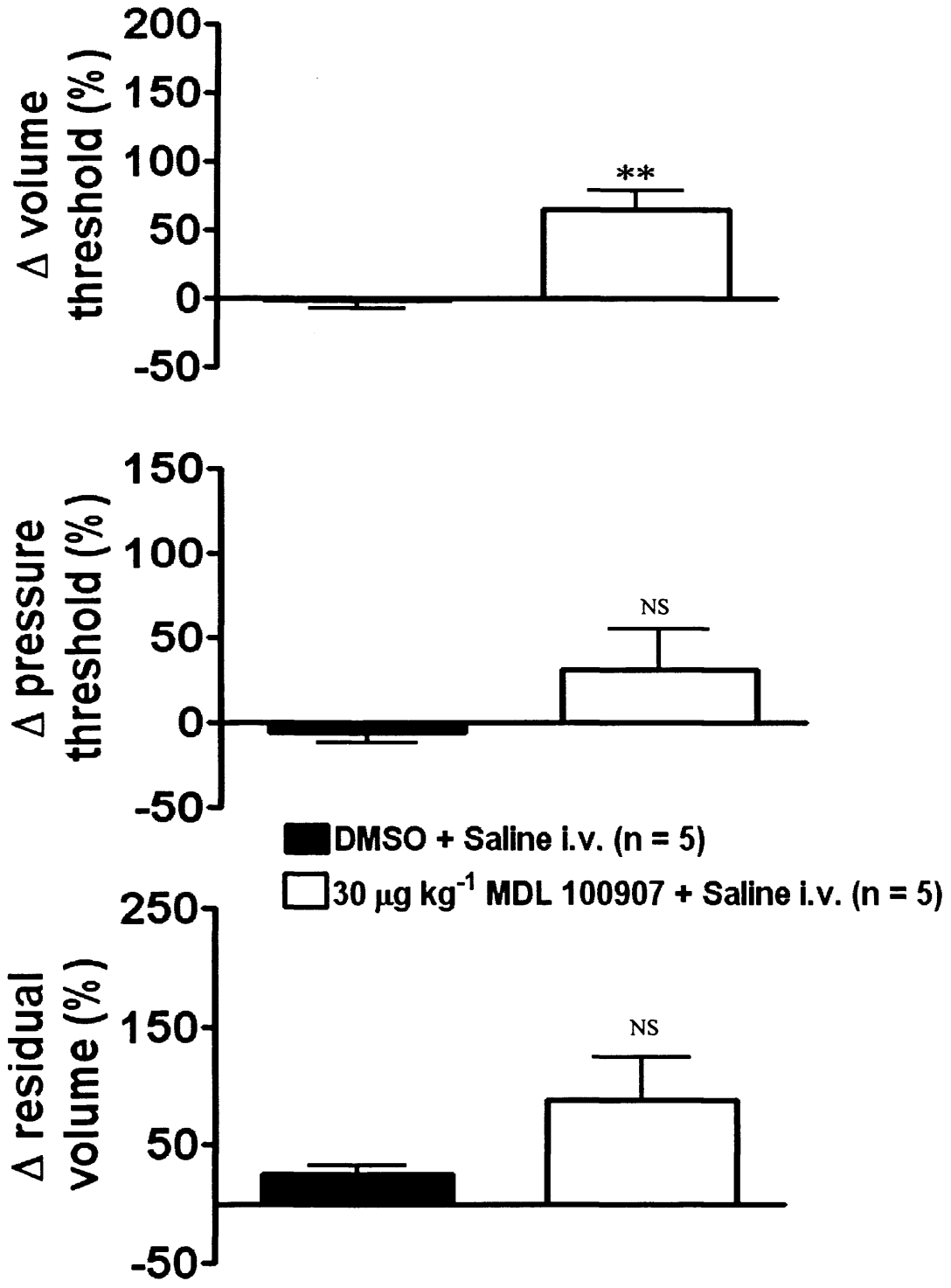

Figure 3.9b Urethane anaesthetised female rats: a comparison of the effects of MDL 100907 and vehicle (saline) on percentage changes (Δ) in volume threshold, pressure threshold and residual volume. Each bar represents mean value and vertical bars show the s.e.mean. Changes caused by MDL 100907 and saline were compared with DMSO + saline control using Student's unpaired t test. ** $P<0.01$, NS non-significant.

3.2.4 Effect of 5-HT_2 receptor antagonists on agonist responses

3.2.4.1 Mianserin (5-HT_2) and WAY 161503 (5-HT_{2C})

Mianserin (100 and 300 μg kg^{-1}, i.v; n = 5) blocked the effects of WAY 161503 (n = 5) on baseline EUS-EMG signal. The low dose of mianserin failed to block the effects of WAY 161503 on urethral pressure whereas the high dose prevented the WAY 161503 evoked increase on urethral pressure (Figure 3.10a).

On the micturition reflex, only the high dose (300 μg kg^{-1}) of mianserin significantly attenuated the ability of WAY 161503 to increase residual volume (12 ± 23%), but not the increases in volume and pressure threshold (Figure 3.10b).

Both doses of mianserin (100 and 300 μg kg^{-1}) significantly attenuated the pressor effect of WAY 161503 on MAP (8 ± 1 and 7 ± 1 mmHg respectively compared with 21 ± 1 mmHg). HR was unaffected.

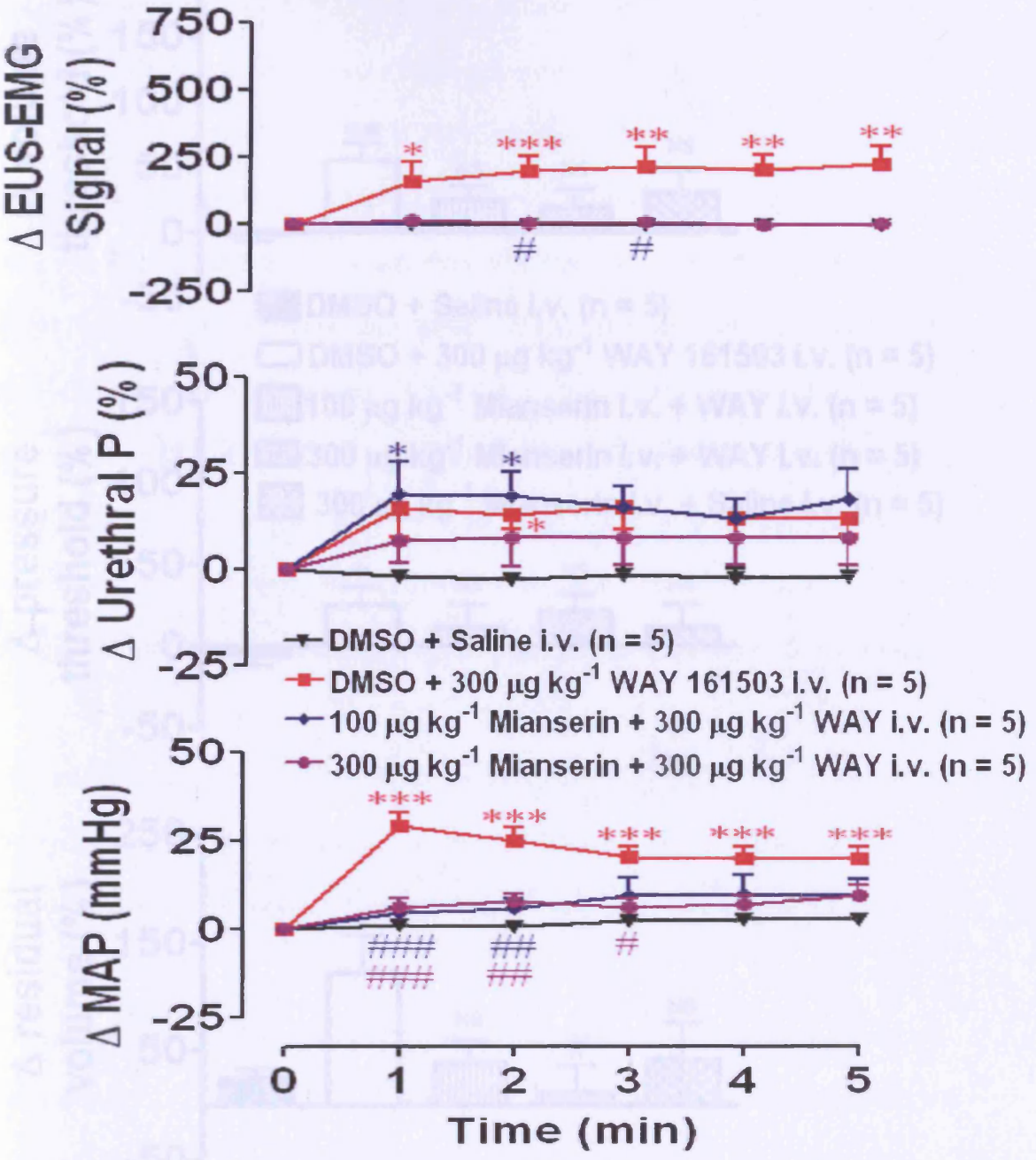

Figure 3.10a Urethane anaesthetised female rats: a comparison of the effects of vehicle (DMSO + saline) and pre-treatment of WAY 161503 with mianserin on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (P) and mean arterial blood pressure (MAP). Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by mianserin and WAY 161503 were compared with DMSO + saline control using two-way analysis of variance followed by the least significant difference test. *,# P<0.05, **,## P<0.01, ***,### P<0.001. (*), compared to DMSO + Saline, (#), compared to DMSO + WAY 161503.

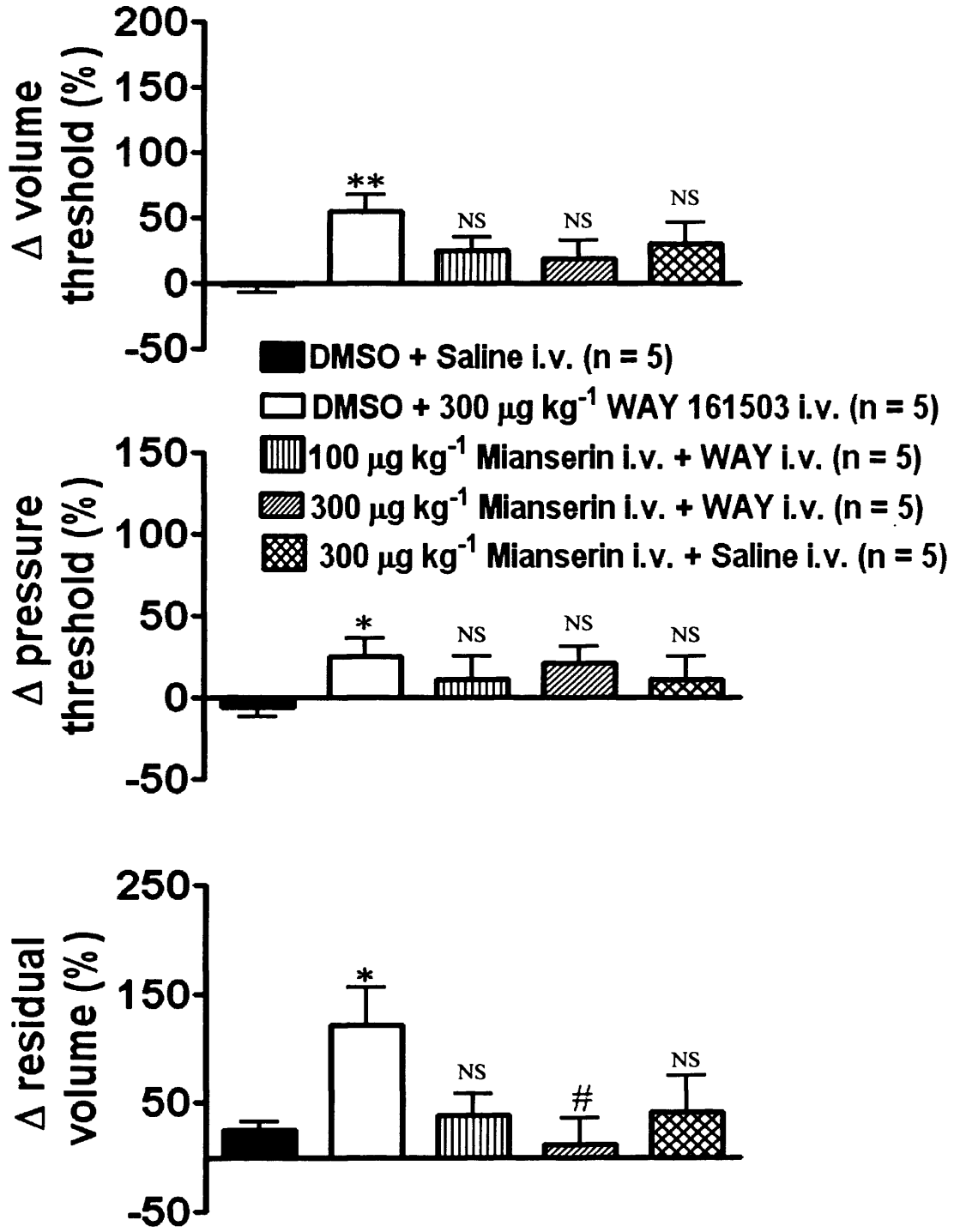

Figure 3.10b Urethane anaesthetised female rats: a comparison of the effects of vehicle (DMSO + saline) and pre-treatment of WAY 161503 with mianserin on percentage changes (Δ) in volume threshold, pressure threshold and residual volume. Each bar represents mean value and vertical bars show the s.e.mean. Changes caused by mianserin and WAY 161503 were compared with DMSO + saline control using Student's unpaired t test. *,# $P<0.05$, ** $P<0.01$, NS non-significant. (*), compared to DMSO + Saline, (#), compared to DMSO + WAY 161503.

3.2.4.2 RS 102221 (5-HT_{2C}) and WAY 161503 (5-HT_{2C})

RS 102221 (30 μg kg^{-1}, i.v; n = 3) blocked the effects of WAY 161503 (n = 3) on baseline EUS-EMG activity, whereas in the presence of the high dose of RS 102221 (500 μg kg^{-1}; i.v; n = 5), the effects of WAY 161503 tended to be potentiated, although non significant when compared to WAY 161503 alone (299 ± 36%). RS 102221 (100 μg kg^{-1}, i.v; n = 4) also failed to block the effects of WAY 161503 on baseline EUS-EMG activity (Figure 3.11a). All three doses of RS 102221 failed to block the WAY 161503 evoked increase in urethral pressure (14 ± 2%, 8 ± 1% and 11 ± 1%, respectively). Onset of appearance of EUS-EMG firing following administration of WAY 161503 pre-treated with RS 102221 (100 and 500 μg kg^{-1}) was 23 ± 5 and 26 ± 5s respectively and again this increase in EUS-EMG activity was observed to be ongoing up to 10 min, where on emptying the bladder to begin testing the micturition reflex, EUS-EMG firing stopped.

For the micturition reflex, only the highest dose of RS 102221 (500 μg kg^{-1}) significantly decreased the effects of WAY 161503 on volume threshold (4 ± 12%), and significantly reversed the increase in residual volume to a decrease (-14 ± 23%; Figure 3.11b).

Only the highest dose of RS 102221 (500 μg kg^{-1}) significantly decreased the pressor effects of WAY 161503 on MAP (7 ± 1 mmHg compared with 21 ± 1 mmHg). HR was unaffected.

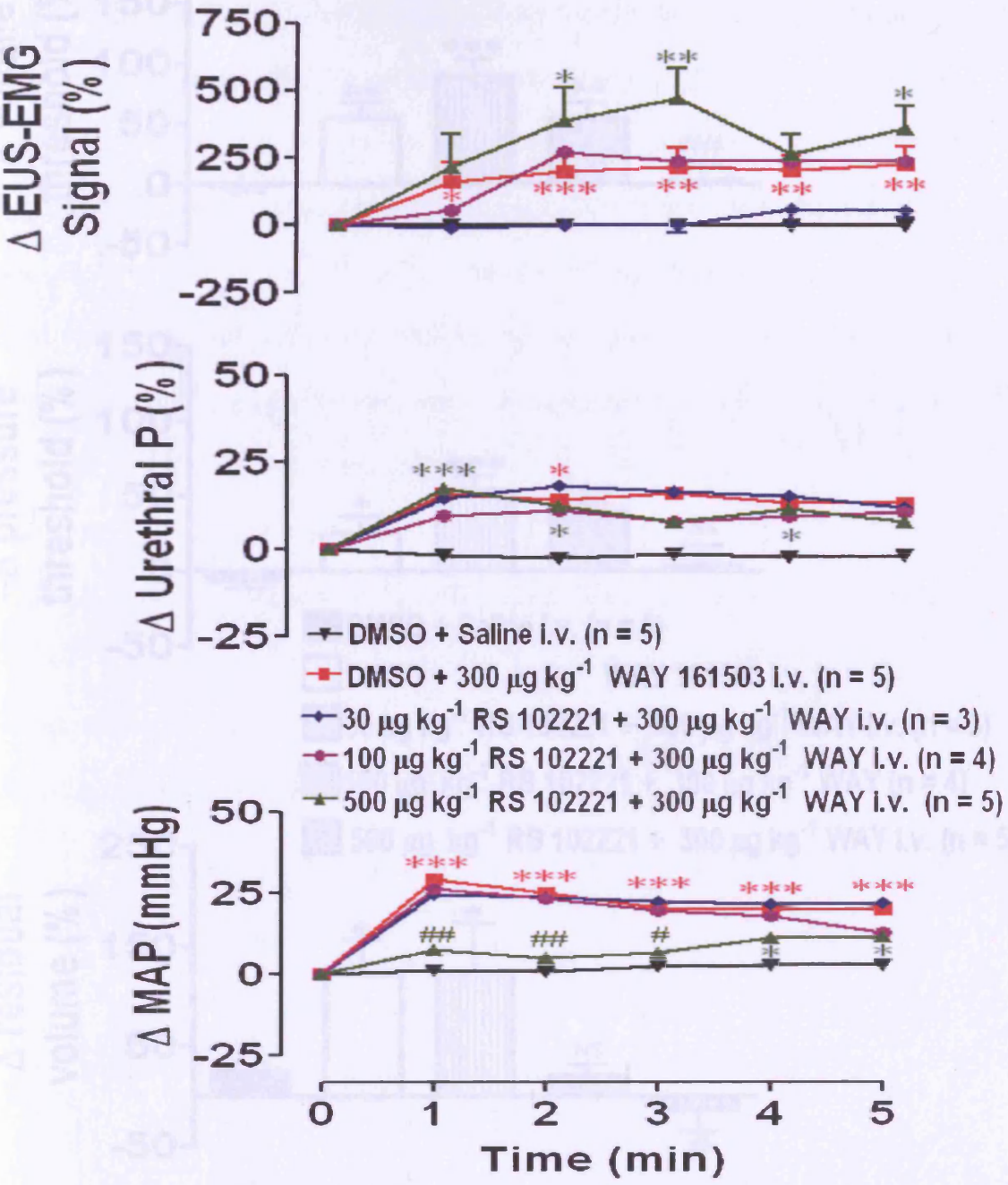

Figure 3.11a Urethane anaesthetised female rats: a comparison of the effects of vehicle (DMSO + saline) and pre-treatment of WAY 161503 with RS 102221 on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (P) and mean arterial blood pressure (MAP). Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by RS 102221 and WAY 161503 were compared with DMSO + saline control using two-way analysis of variance followed by the least significant difference test. *,# $P<0.05$, **,### $P<0.01$, *** $P<0.001$. (*), compared to DMSO + Saline, (#), compared to DMSO + WAY 161503.

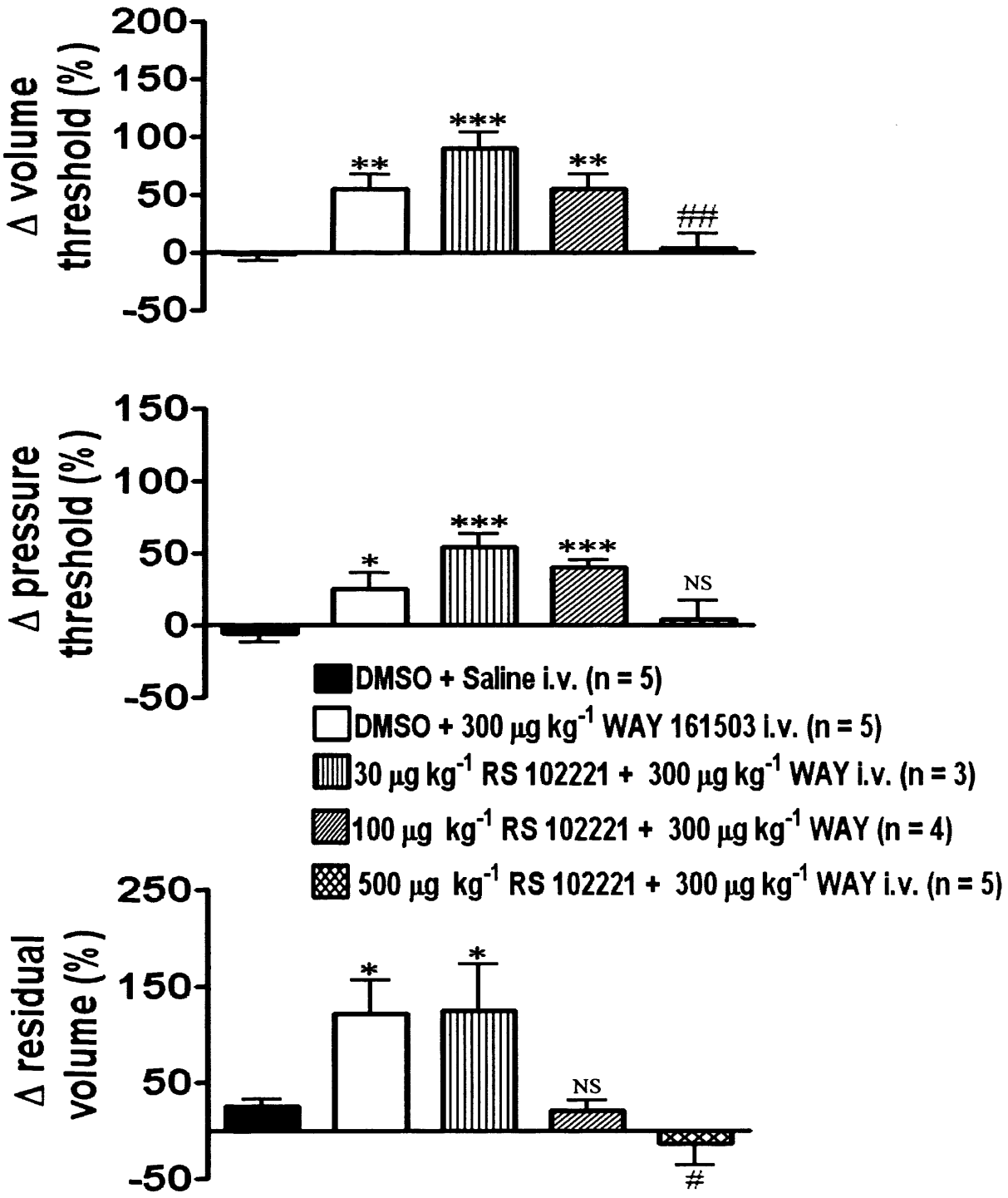

Figure 3.11b Urethane anaesthetised female rats: a comparison of the effects of vehicle (DMSO + saline) and pre-treatment of WAY 161503 with RS 102221 on percentage changes (Δ) in volume threshold, pressure threshold and residual volume. Each bar represents mean value and vertical bars show the s.e.mean. Changes caused by RS 102221 and WAY 161503 were compared with DMSO + saline control using Student's unpaired t test. *,# $P<0.05$, **,## $P<0.01$, ***$P<0.001$, NS non-significant. (*), compared to DMSO + Saline, (#), compared to DMSO + WAY 161503.

3.2.4.3 RS 102221 (5-HT_{2C}) and mCPP (5-HT_{2C})

RS 102221 (500 μg kg^{-1}, i.v; n = 5) failed to block the effects of mCPP (n = 5) on baseline EUS-EMG signal (533 ± 84%) and urethral pressure (19 ± 3%; Figure 3.12a). The effects observed on EUS-EMG activity following pre-treatment of mCPP with RS 102221 tended to be potentiated, although non significant when compared to mCPP alone. Onset of appearance of EUS-EMG firing following administration of mCPP pre-treated with RS 102221 was 11 ± 3s and this evoked increase in EUS-EMG activity was observed to be ongoing up to 10 min, where on emptying the bladder to begin testing the micturition reflex EUS-EMG firing stopped.

RS 102221 failed to interfere with the ability of mCPP to block the micturition reflex.

Pre-treatment of mCPP with RS 102221 now caused a significant decrease in MAP (-15 ± 2 mmHg; Figure 3.12a). HR was unaffected.

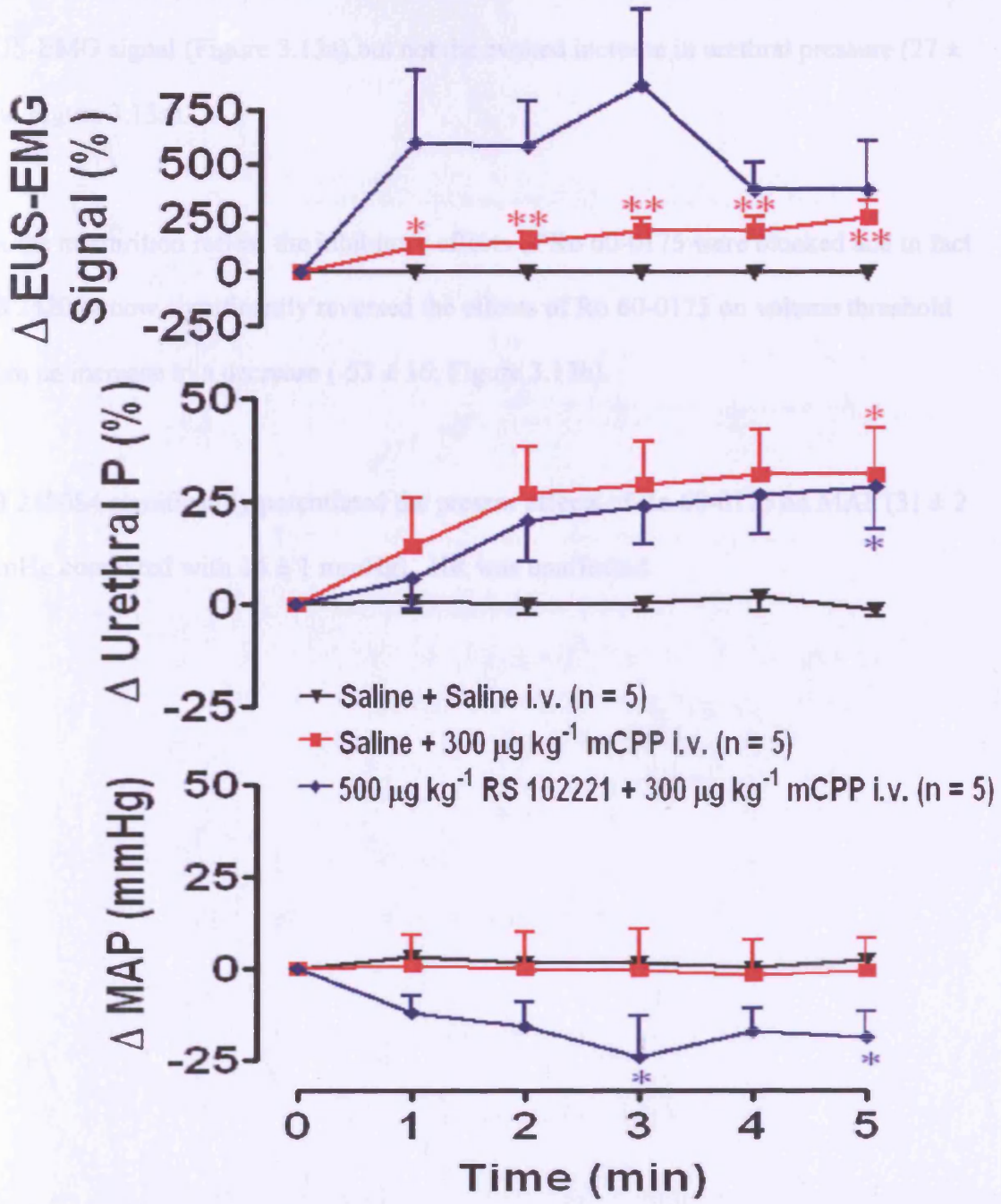

Figure 3.12a Urethane anaesthetised female rats: a comparison of the effects of vehicle (DMSO + saline) and pre-treatment of mCPP with RS 102221 on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (UP) and mean arterial blood pressure (MAP). Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by RS 102221 and mCPP were compared with DMSO + saline control using two-way analysis of variance followed by the least significant difference test. * $P<0.05$, ** $P<0.01$.

3.2.4.4 SB 242084 (5-HT_{2C}) and Ro 60-0175 (5-HT_{2C})

SB 242084 (30 μg kg^{-1}, i.v; n = 5) blocked the effects of Ro 60-0175 (n = 5) on baseline EUS-EMG signal (Figure 3.13a) but not the evoked increase in urethral pressure (27 ± 3%; Figure 3.13a).

On the micturition reflex, the inhibitory effects of Ro 60-0175 were blocked and in fact SB 242084 now significantly reversed the effects of Ro 60-0175 on volume threshold from an increase to a decrease (-53 ± 16; Figure 3.13b).

SB 242084 significantly potentiated the pressor effects of Ro 60-0175 on MAP (31 ± 2 mmHg compared with 14 ± 1 mmHg). HR was unaffected.

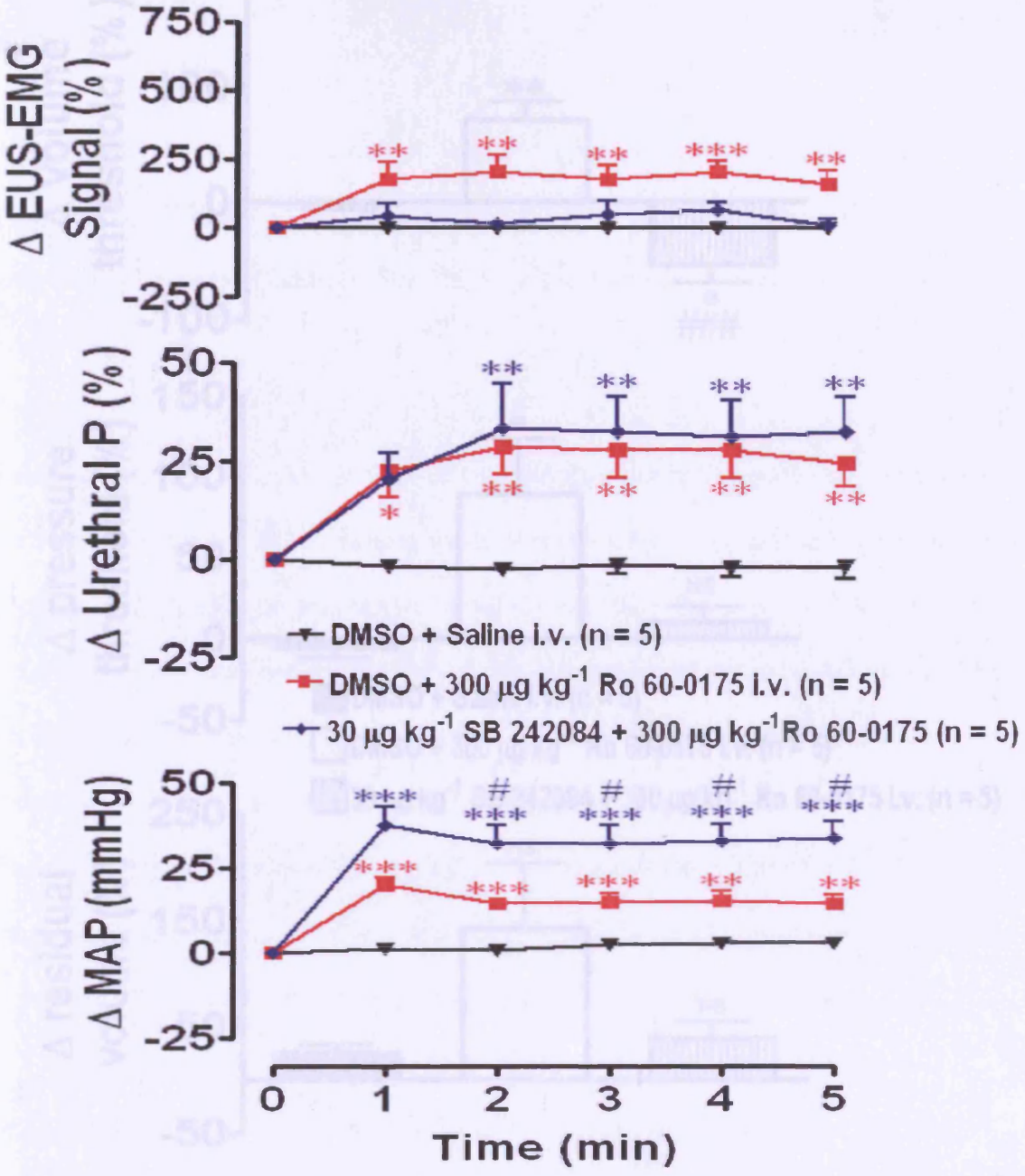

Figure 3.13a Urethane anaesthetised female rats: a comparison of the effects of vehicle (DMSO + saline) and pre-treatment of Ro 60-0175 with SB 242084 on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (UP) and mean arterial blood pressure (MAP). Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by SB 242084 and Ro 60-0175 were compared with DMSO + saline control using two-way analysis of variance followed by the least significant difference test. *,# P<0.05, ** P<0.01, ***, P<0.001. (*), compared to DMSO + Saline, (#), compared to DMSO + Ro 60-0175.

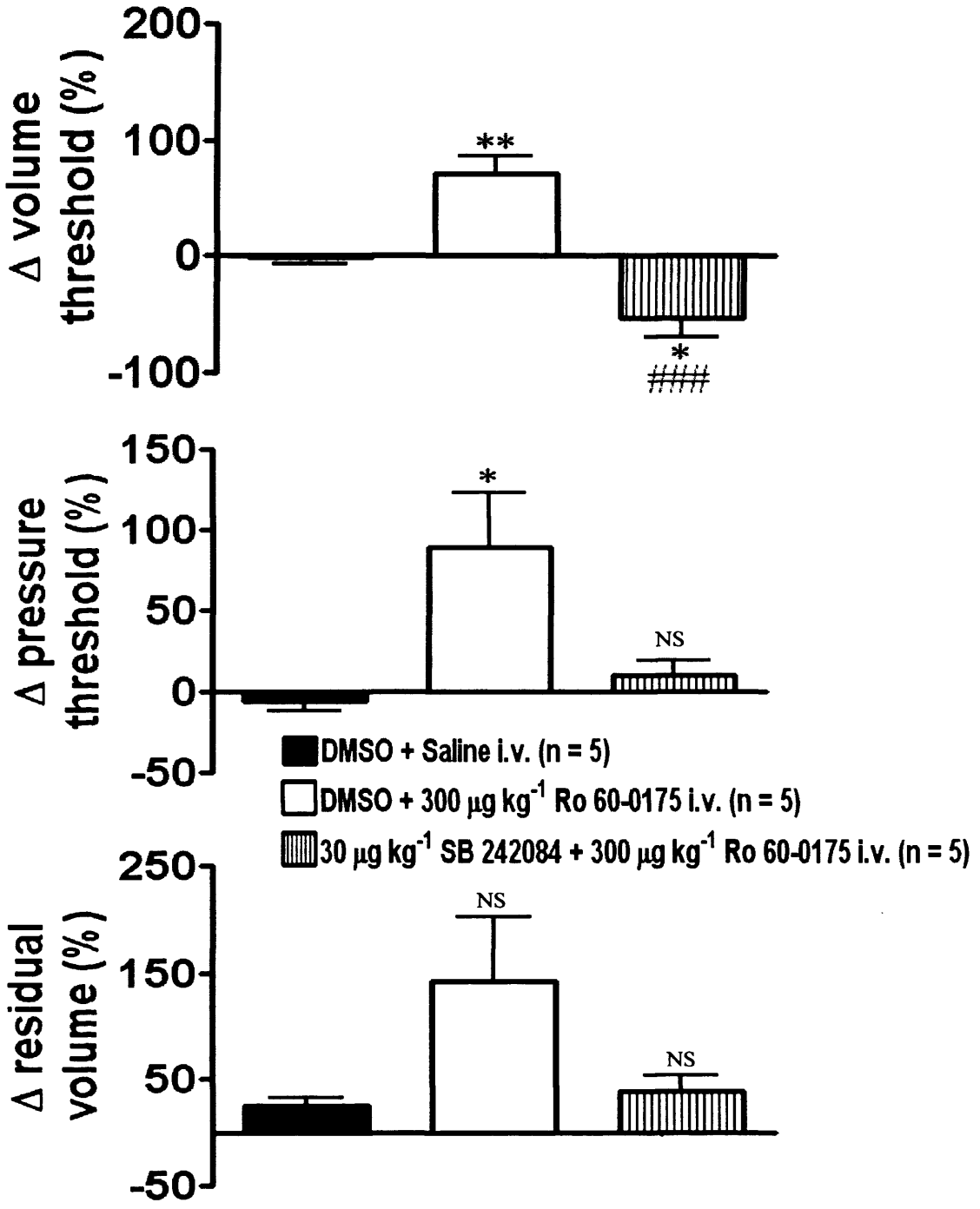

Figure 3.13b Urethane anaesthetised female rats: a comparison of the effects of vehicle (DMSO + saline) and pre-treatment of Ro 60-0175 with SB 242084 on percentage changes (Δ) in volume threshold, pressure threshold and residual volume. Each bar represents mean value and vertical bars show the s.e.mean. Changes caused by SB 242084 and Ro 60-0175 were compared with DMSO + saline control using Student's unpaired t test. *, $P<0.05$, ** $P<0.01$, ###, $P<0.001$, NS non-significant. (*), compared to DMSO + Saline, (#), compared to DMSO + Ro 60-0175.

3.2.4.5 Ketanserin & MDL 100907 (5-HT_{2A}) and WAY 161503 (5-HT_{2C})

Pre-treatment with either ketanserin (30 and 100 μg kg^{-1}, i.v; n = 5) or MDL 100907 (30 μg kg^{-1}, i.v; n = 5) blocked the effects of WAY 161503 (n = 5) on baseline EUS-EMG signal but not on urethral pressure (9 ± 1%, 14 ± 1% and 7 ± 1% respectively; Figure 3.14a).

On the micturition reflex, the low dose of ketanserin (30 μg kg^{-1}) significantly potentiated the effects of WAY 161503 on volume threshold (153 ± 33%) and tended to increase the effects of WAY 161503 on pressure threshold (61 ± 17%). The effect of WAY 161503 on residual volume now became highly variable and was found not to be significant. The high dose of ketanserin (100 μg kg^{-1}) failed to block the effects of WAY 161503 on volume threshold (81 ± 13%), pressure threshold (53 ± 14%) and residual volume (92 ± 18%). Similarly, MDL 100907 (30 μg kg^{-1}) failed to block the effects of WAY 161503 on volume threshold (92 ± 23%) and pressure threshold (83 ± 24%) and again the effects on residual volume were again highly variable (see Figure 3.14b for graphs).

The low dose (30 μg kg^{-1}) of ketanserin failed to attenuate the pressor (25 ± 3 mmHg) effect of WAY 161503 on MAP whereas the high dose (100 μg kg^{-1}) and 30 μg kg^{-1} dose of MDL 100907 significantly attenuated the pressor response evoked by WAY 161503 on MAP (5 ± 1 mmHg and 4 ± 1 mmHg respectively compared with 21 ± 1 mmHg; Figure 3.14a). HR was unaffected.

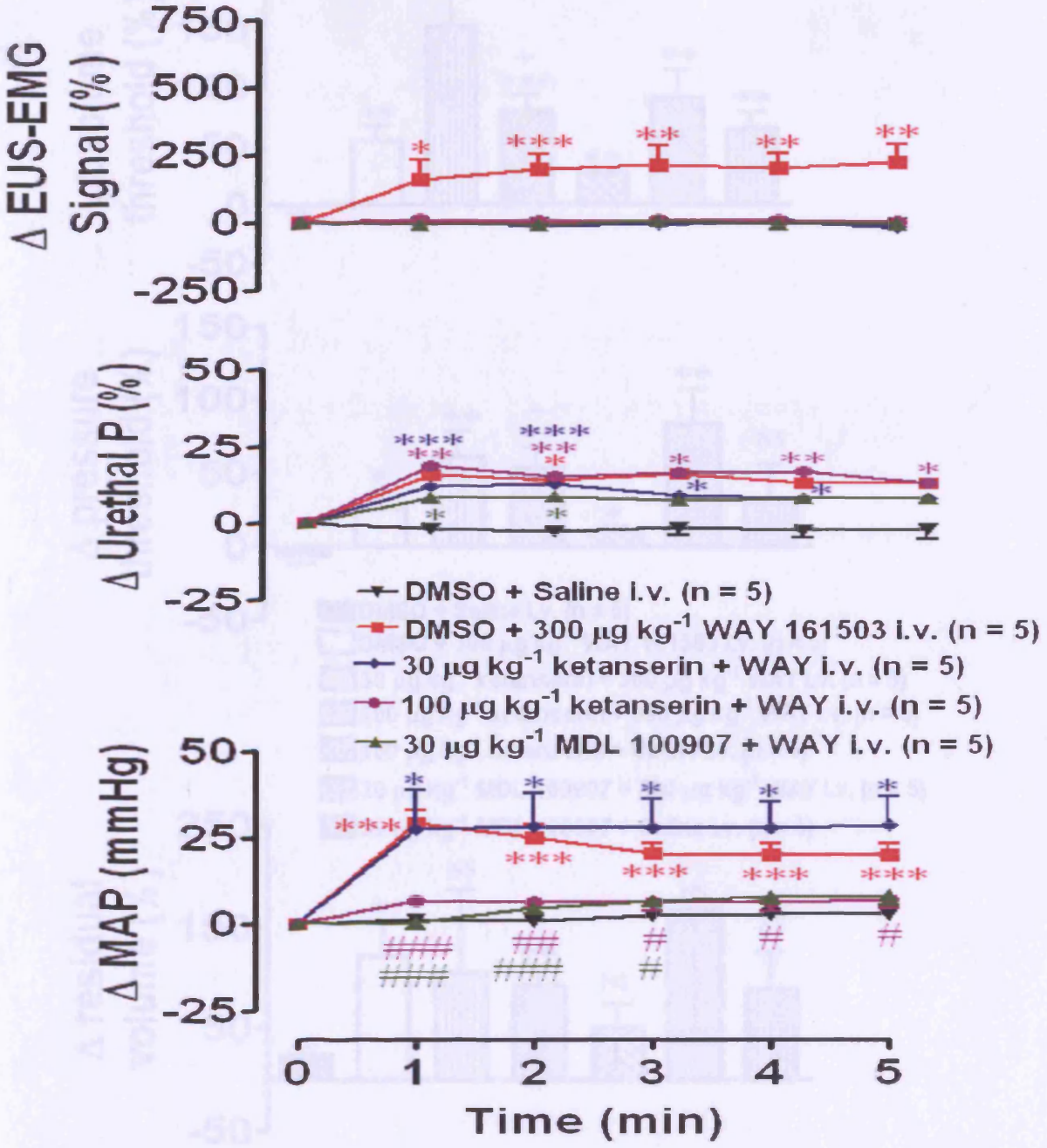

Figure 3.14a Urethane anaesthetised female rats: a comparison of the effects of vehicle (DMSO + saline) and pre-treatment of WAY 161503 with ketanserin or MDL 100907 on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (UP) and mean arterial blood pressure (MAP). Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by ketanserin, MDL 100907 and WAY 161503 were compared with DMSO + saline control using two-way analysis of variance followed by the least significant difference test. *,# $P<0.05$, **,## $P<0.01$, ***,### $P<0.001$. (*), compared to DMSO + Saline, (#), compared to DMSO + WAY 161503.

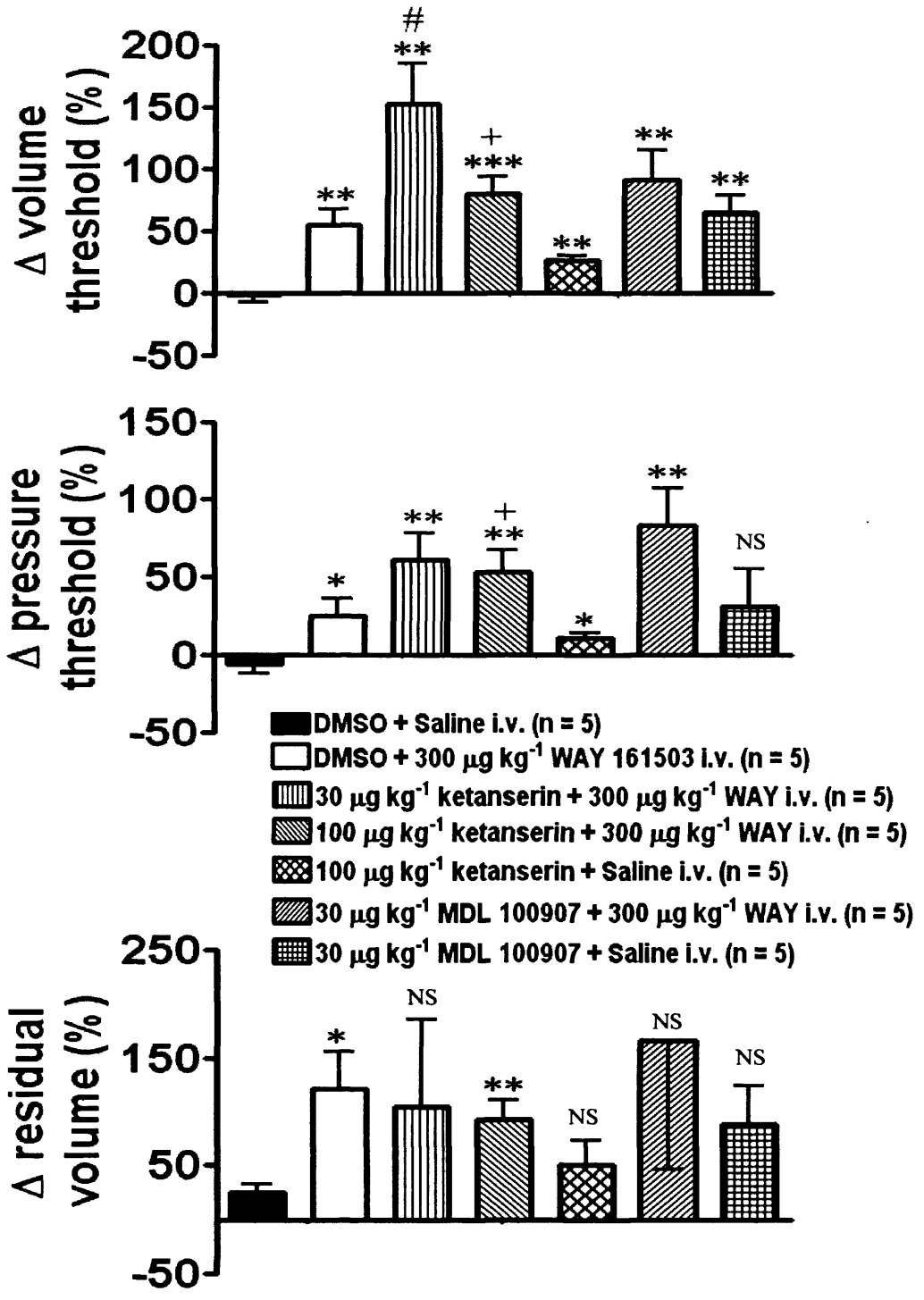

Figure 3.14b Urethane anaesthetised female rats: a comparison of the effects of vehicle (DMSO + saline) and pre-treatment of WAY 161503 with ketanserin or MDL 100907 on percentage changes (Δ) in volume threshold, pressure threshold and residual volume. Each bar represents mean value and vertical bars show the s.e.mean. Changes caused by ketanserin, MDL 100907 and WAY 161503 were compared with DMSO + saline control using Student's unpaired t test. *,#,+ $P<0.05$, ** $P<0.01$, *** $P<0.001$, NS non-significant. (*), compared to DMSO + Saline, (#), compared to DMSO + WAY 161503. (+), compared to ketanserin + saline.

3.2.4.6 Ketanserin & MDL 100907 (5-HT_{2A}) and Ro 60-0175 (5-HT_{2C})

Pre-treatment with either ketanserin or MDL 100907 (30 μg kg^{-1}, i.v; n = 5) blocked the effects of Ro 60-0175 (n = 5) on baseline EUS-EMG signal but not urethral pressure (9 ± 1 and 11 ± 2 % respectively compared with 23 ± 2; Figure 3.15a).

On the micturition reflex, both ketanserin and MDL 100907 failed to block the inhibitory effects of Ro 60-0175 on volume threshold (61 ± 5 and 63 ± 6% compared with 71 ± 15%) and pressure threshold (50 ± 3 and 44 ± 6% compared with 90 ± 33%; Figure 3.15b). The tendency of Ro 60-0175 to increase residual volume was now non existent.

Both ketanserin and MDL 100907 failed to block the pressor effect of Ro 60-0175 on MAP (16 ± 2 and 24 ± 1 mmHg respectively compared with 14 ± 1 mmHg). HR was unaffected.

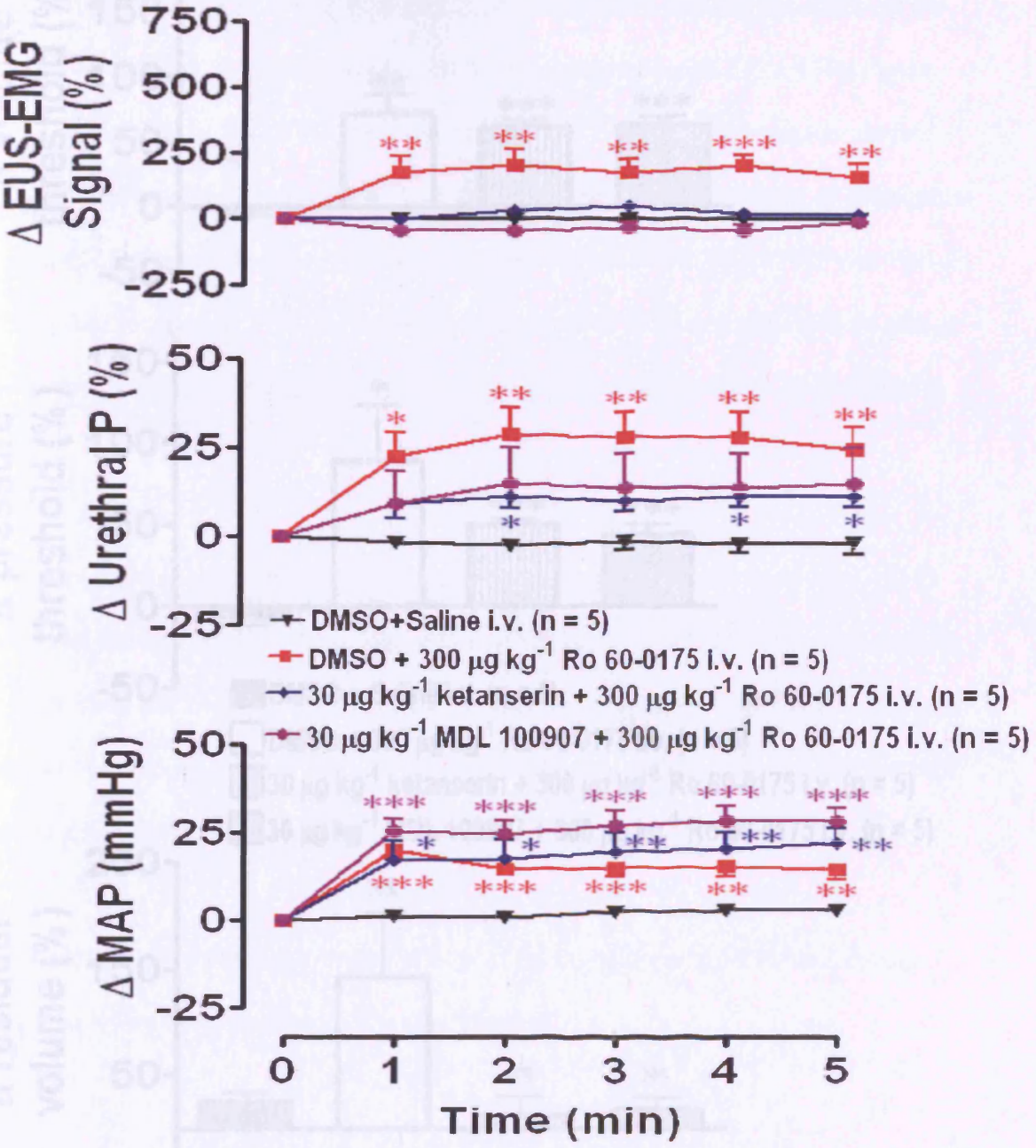

Figure 3.15a Urethane anaesthetised female rats: a comparison of the effects of vehicle (DMSO + saline) and pre-treatment of Ro 60-0175 with ketanserin or MDL 100907 on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (UP) and mean arterial blood pressure (MAP). Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by ketanserin, MDL 100907 and Ro 60-0175 were compared with DMSO + saline control using two-way analysis of variance followed by the least significant difference test. * P<0.05, ** P<0.01, *** P<0.001.

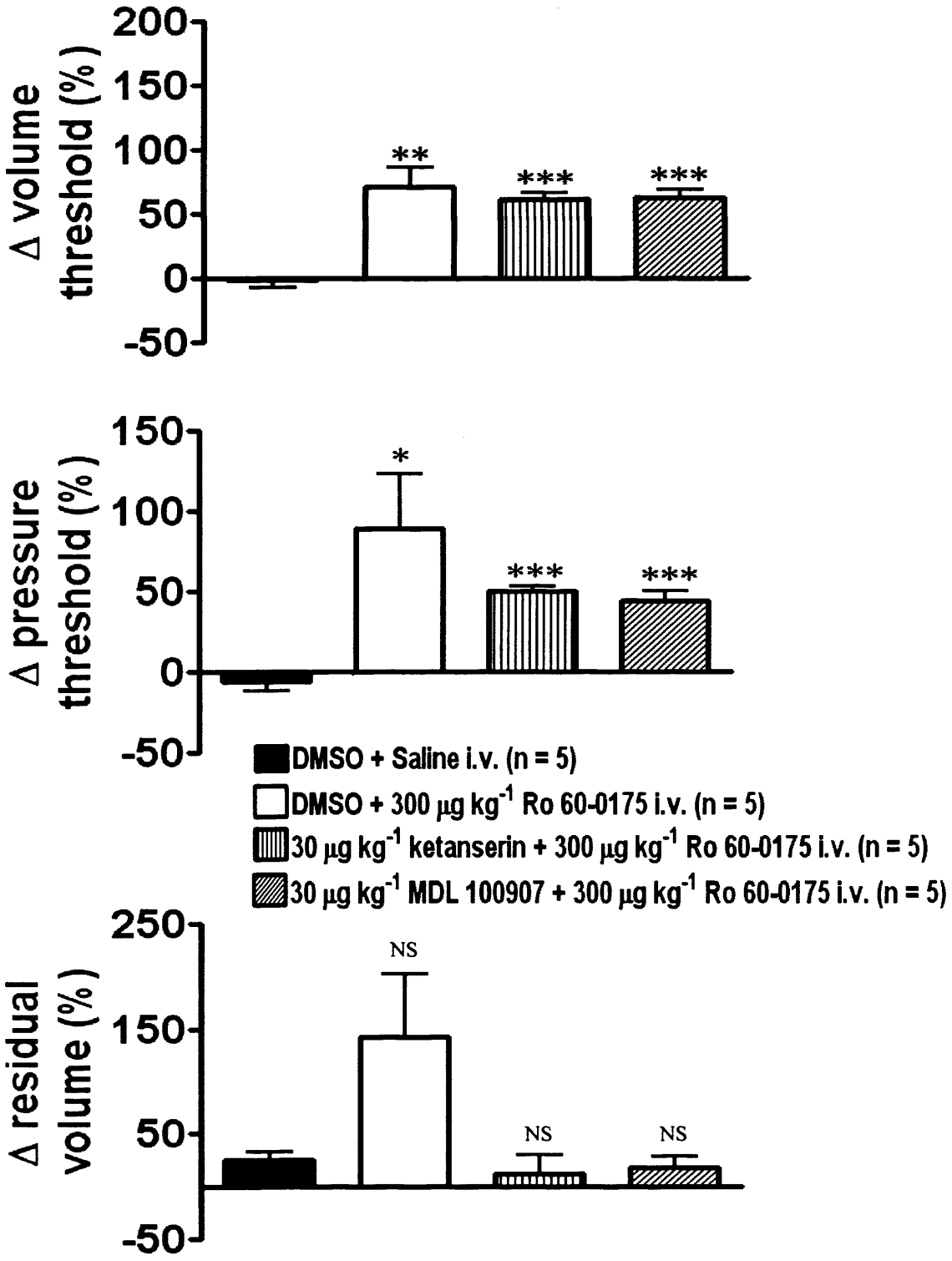

Figure 3.15b Urethane anaesthetised female rats: a comparison of the effects of vehicle (DMSO + saline) and pre-treatment of Ro 60-0175 with ketanserin or MDL 100907 on percentage changes (Δ) in volume threshold, pressure threshold and residual volume. Each bar represents mean value and vertical bars show the s.e.mean. Changes caused by ketanserin, MDL 100907 and Ro 60-0175 were compared with DMSO + saline control using Student's unpaired t test. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, NS non-significant.

3.2.4.7 RS 127445 (5-HT_{2B}) and Ro 60-0175 (5-HT_{2C})

Pre-treatment with RS 127445 (300 μg kg^{-1}, i.v; n = 5) failed to block the effects of Ro 60-0175 on baseline EUS-EMG signal (218 ± 30% compared with 179 ± 17%; Figure 3.16a). Interestingly, RS 127445 reversed the Ro 60-0175 evoked increase in urethral pressure to a non significant decrease (-11 ± 4%; Figure 3.16a). The onset of appearance of EUS-EMG firing following pre-treatment of Ro 60-0175 with RS 127445 was 19 ± 2s. This evoked increase in EUS-EMG activity was observed to be ongoing up to 10 min at which time the bladder was emptied to test the micturition reflex and EUS-EMG firing stopped.

On the micturition reflex, RS 127445 failed to have a significant effect on Ro 60-0175 evoked responses on both volume and pressure threshold. Interestingly, even though Ro 60-0175 alone had no significant effect on residual volume due to high variability, pre-treatment of with RS 127445 reduced this variability, thus causing a significant increase in residual volume (64 ± 11%; Figure 3.16b).

RS 127445 failed to block the pressor effects of Ro 60-0175 on MAP (25 ± 2 mmHg compared with 14 ± 1 mmHg). HR was unaffected.

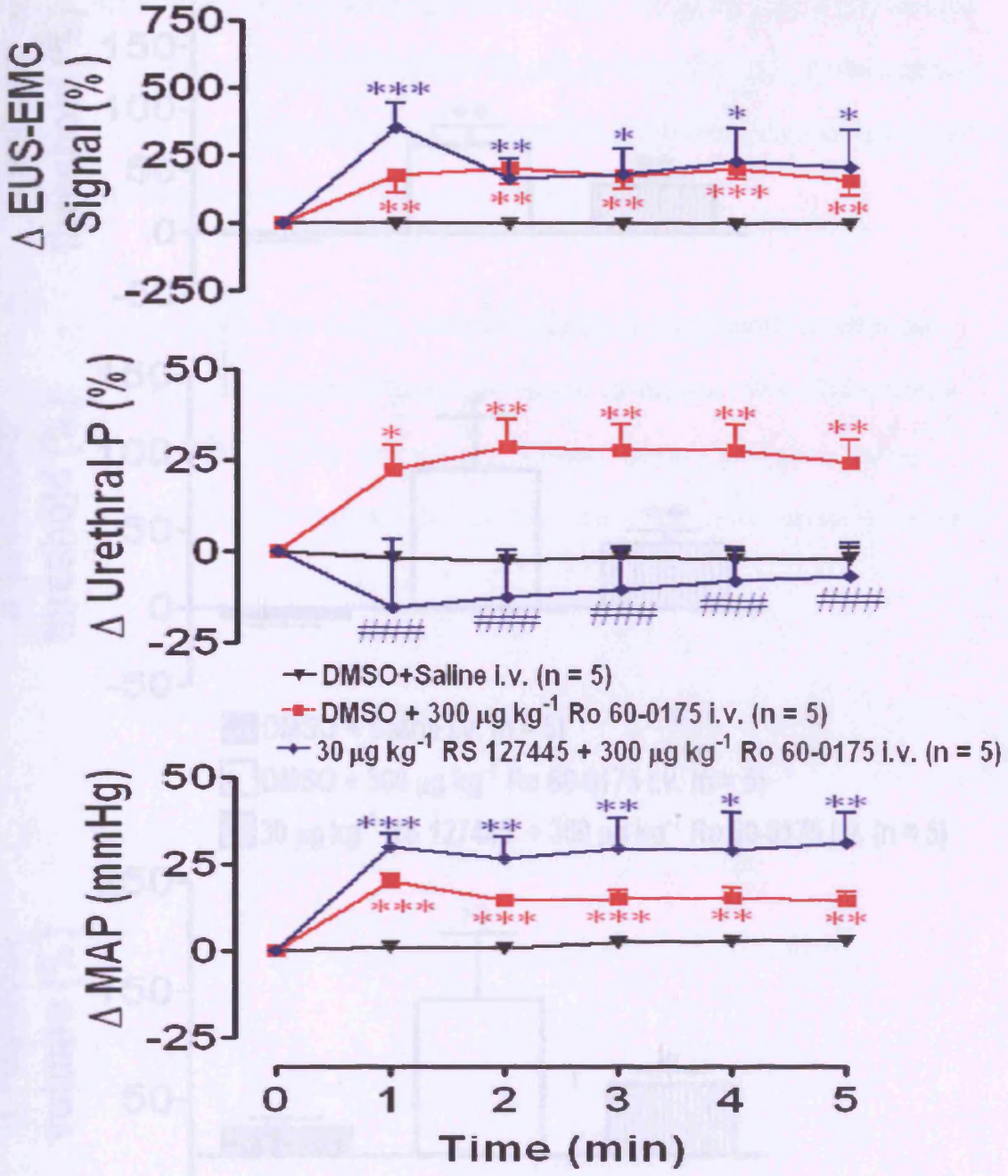

Figure 3.16a Urethane anaesthetised female rats: a comparison of the effects of vehicle (DMSO + saline) and pre-treatment of Ro 60-0175 with RS 127445 on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (UP) and mean arterial blood pressure (MAP). Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by RS 127445 and Ro 60-0175 were compared with DMSO + saline control using two-way analysis of variance followed by the least significant difference test. * P<0.05, ** P<0.01, ***,### P<0.001. (*), compared to DMSO + Saline, (#), compared to DMSO + Ro 60-0175.

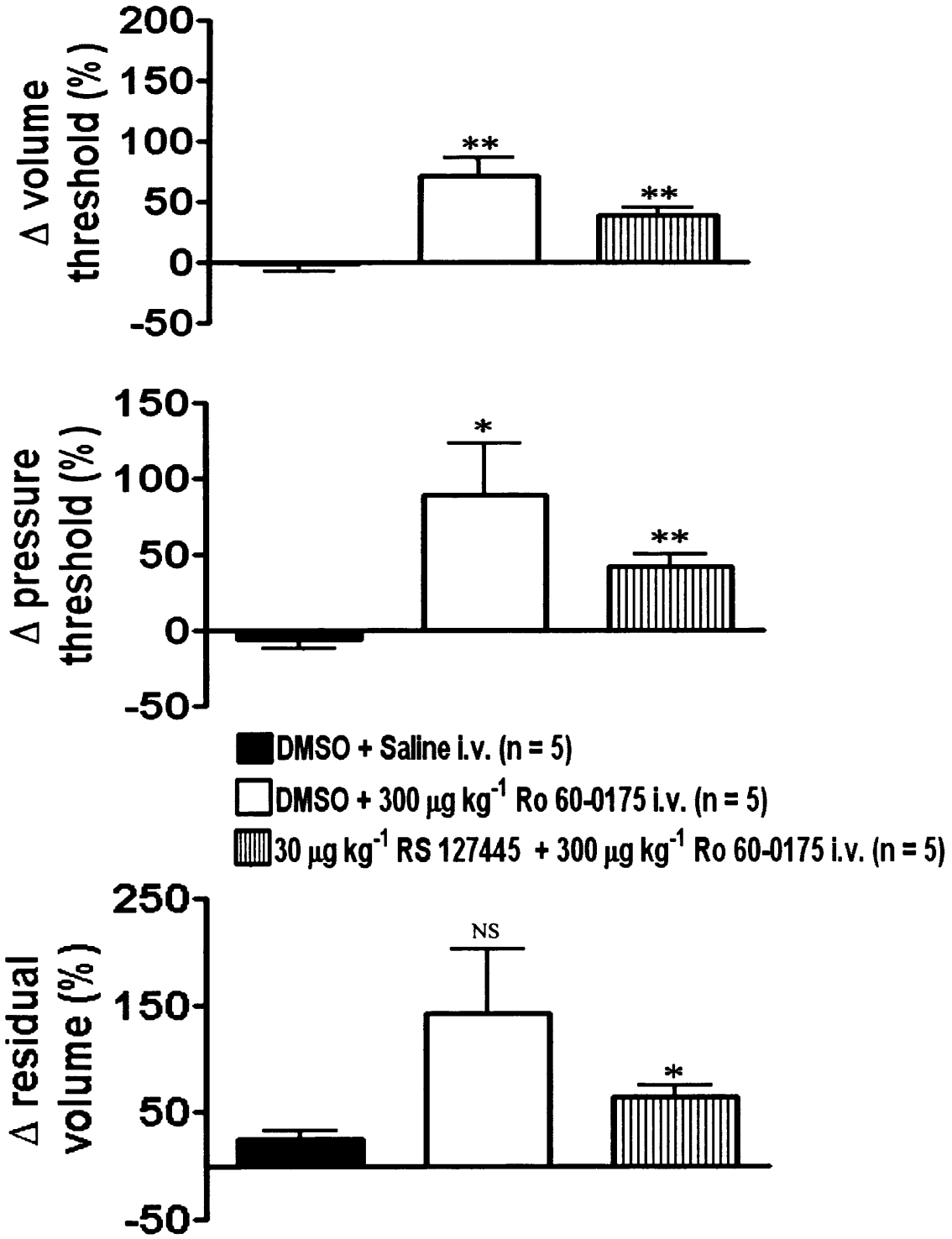

Figure 3.16b Urethane anaesthetised female rats: a comparison of the effects of vehicle (DMSO + saline) and pre-treatment of Ro 60-0175 with RS 127445 on percentage changes (Δ) in volume threshold, pressure threshold and residual volume. Each bar represents mean value and vertical bars show the s.e.mean. Changes caused by RS 127445 and Ro 60-0175 were compared with DMSO + saline control using Student's unpaired t test. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, NS non-significant.

3.2.4.8 Ketanserin & MDL 100907 (5-HT_{2A}) and DOI (5-HT_{2A})

Both ketanserin (30 μg kg^{-1}, i.v; n = 5) and MDL 100907 (30 μg kg^{-1}, i.v; n = 3) blocked the ability of DOI (n = 3/5) to evoke EUS-EMG activity (Figure 3.17a). Pre-treatment of DOI with the 5-HT_{2A} receptor antagonists now tended to increase urethral pressure, but these increases were found not to be significant (Figure 3.17a).

On the micturition reflex, both 5-HT_{2A} receptor antagonists significantly reversed the effects of DOI on volume threshold from a decrease to an increase (43 ± 15% and 90 ± 43% respectively; Figure 3.17b). Interestingly, administration of DOI alone had no significant effect on pressure threshold but pre-treatment of DOI with ketanserin caused a significant increase of 63 ± 22% (Figure 3.17b).

Ketanserin and MDL 100907 failed to interfere with the pressor effect evoked by DOI on MAP (14 ± 1 mmHg and 24 ± 3 mmHg respectively compared with 21 ± 1 mmHg; Figure 3.17a). HR was unaffected.

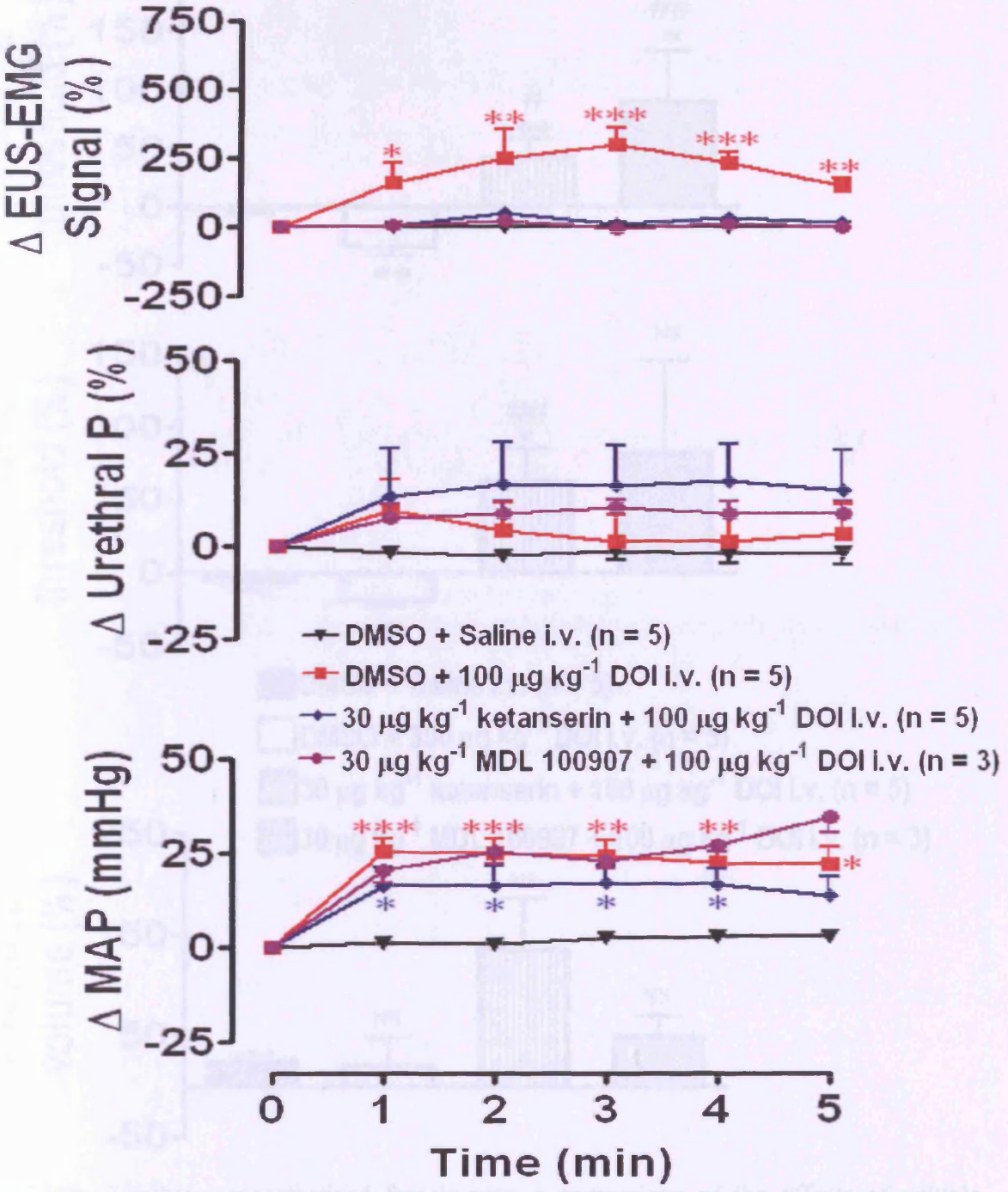

Figure 3.17a Urethane anaesthetised female rats: a comparison of the effects of vehicle (DMSO + saline) and pre-treatment of DOI with ketanserin or MDL 100907 on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (UP) and mean arterial blood pressure (MAP). Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by ketanserin, MDL 100907 and DOI were compared with DMSO + saline control using two-way analysis of variance followed by the least significant difference test. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

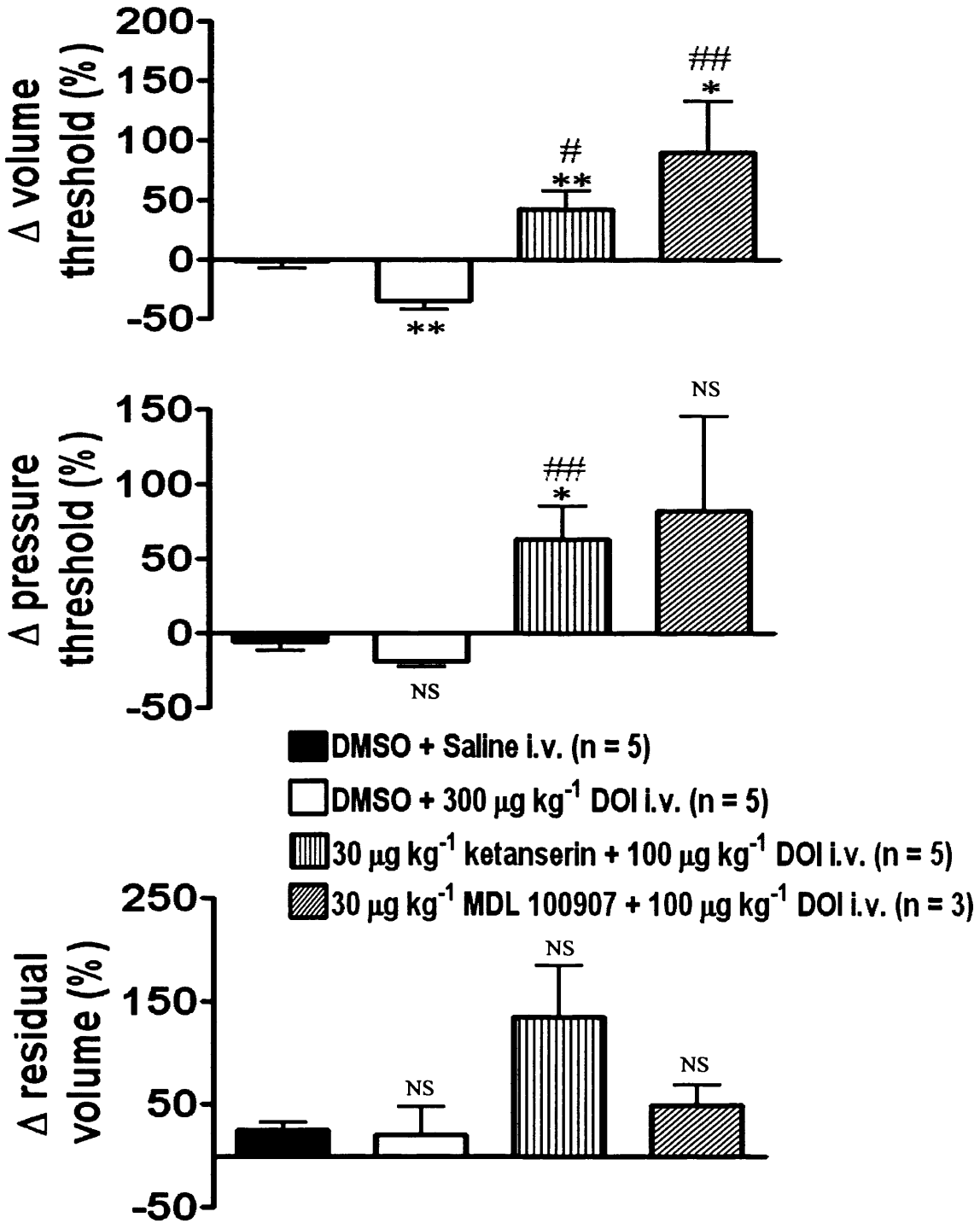

Figure 3.17b Urethane anaesthetised female rats: a comparison of the effects of vehicle (DMSO + saline) and pre-treatment of DOI with ketanserin or MDL 100907 on percentage changes (Δ) in volume threshold, pressure threshold and residual volume. Each bar represents mean value and vertical bars show the s.e.mean. Changes caused by ketanserin, MDL 100907 and DOI were compared with DMSO + saline control using Student's unpaired t test. *,# $P<0.05$, **,## $P<0.01$, NS non-significant. (*), compared to DMSO + Saline, (#), compared to DMSO + DOI.

300 μg kg^{-1} Ro 60-0175 bolus i.v. (n = 3)		
Sample no.	**Time (min)**	**Measured [Free Plasma] nM**
1 (C_{max})	0.5	**49**
2	10	**15**

Table 3.1 Summary of measured free plasma concentrations of 5-HT_{2C} receptor agonist Ro 60-0175. Blood samples were taken at 30s and 10min following administration of Ro 60-0175.

	selectivity				Micturition reflex				Cardiovascular effects	
					Overall	Vol Thres	Pressure Thres	Residual Vol	Blood Pressure	Heart Rate
WAY161503	5-HT_{2C}	agonist	↑↑↑	↑ (13)	inhibited	↑↑	↑↑	↑↑↑	↑↑	↔
Ro 60-0175	5-HT_{2C}	agonist	↑↑↑	↑↑ (23)	inhibited	↑↑	↑↑	↔	↑ (14)	↓ (13)
mCPP	5-HT_{2C}	agonist	↑↑↑	↑↑ (24)	abolished	XXXX	XXXX	XXXX	↔	↔
DOI	5-HT_{2A}	agonist	↑↑↑	↔	excited	↓	↔	↔	↑↑ (21)	↔
BW723C86	5-HT_{2B}	agonist	↔	↔	inhibited	↔	↑	↔	↔	↔
Mianserin	5$HT_{2A/2B/2C}$	antagonist	↔	↔	no effect	↔	↔	↔	↔↑	↔
Ketanserin	5$HT_{2A/1D}$; α_1 H_1	antagonist	↔	↔	inhibited	↑	↑	↔	↔	↔
MDL100907	5-HT_{2A}	antagonist	↔	↔	inhibited	↑	↔	↔	↑ (14)	↔
WAY161503 Mianserin 100 & 300			blocked	↑	unaffected	↑↑	↑↑	↔	attenuated (8 & 7)	↔
WAY161503 RS102221 (30, 100 & 500)			only low dose blocked	↑ unaffected	block high dose only	↔	↔	↔↓	block high dose only	↔
mCPP RS102221 (500)			no block, tended to potentiate	↑ unaffected	abolished	XXXX	XXXX	XXXX	↓ (-15)	↔
Ro 60-0175 SB 242084	5-HT_{2C}	antagonist	blocked	↑ unaffected	blocked	↓	↔	↔	potentiate ↑↑↑ (31)	↔
WAY161503 Ketanserin 30 Ketanserin 100			blocked blocked	↑ unaffected ↑ unaffected	potentiates no effect	↑↑↑ ↑↑	↑↑ ↑↑	↔ variable ↑↑↑	↑ atten ↔	↔ ↔
WAY161503 MDL1000907			blocked	↑ unaffected	no effect	↑↑	↑↑	↔ variable	atten ↔	↔
Ro 60-0175 Ketanserin 30			blocked	↑ unaffected	no effect	↑↑	↑↑	↔	blocked	↔
Ro 60-0175 MDL 100907			blocked	↑ unaffected	no effect	↑↑	↑↑	↔	blocked	↔
Ro 60-0175 RS 127445	5-HT_{2B}	antagonist	↑↑↑	blocked	no effect	↑↑	↑↑	↔	no effect	↔
DOI Ketanserin 30			blocked	unaffected	reversed	↑	↑↑	↑ variable	↑↑ (14)	↔
DOI MDL100907 30			blocked	unaffected	reversed	↑↑	↑ variable	↔	↑↑ (24)	↔

Table 3.2 Summary of 5-HT_2 receptor agonist and/or antagonist evoked responses on EUS-EMG, urethral pressure, micturition reflex and cardiovascular effects

A

Experimental Group	n	Onset time of EUS-EMG activity (s)
300 μg kg^{-1} WAY 161503 i.v.	5	28 ± 11
300 μg kg^{-1} Ro 60-0175 i.v.	5	36 ± 13
300 μg kg^{-1} mCPP i.v.	5	20 ± 5
30 μg kg^{-1} DOI i.v.	3	48 ± 9
50 μg kg^{-1} DOI i.v.	3	46 ± 6
100 μg kg^{-1} DOI i.v.	5	17 ± 3

B

100 μg kg^{-1} RS 10 2221 + WAY 161503 i.v.	4	23 ± 5
500 μg kg^{-1} RS 10 2221 + WAY 161503 i.v.	5	26 ± 5
500 μg kg^{-1} RS 10 2221 + mCPP i.v.	5	11± 3

C

30 μg kg^{-1} RS 127445 + Ro 60-0175 i.v.	5	19 ± 2

Table 3.3 Summary of onset times of appearance of EUS-EMG activity following **A** WAY 161503, Ro 60-0175, mCPP and DOI (i.v.); **B** pre-treatment of WAY 161503 and mCPP with RS 102221 (i.v.); **C** pre-treatment of Ro 60-0175 with RS 127445 (i.v.). All values are expressed as means ± sem. No statistical differences were observed when comparisons were made between the different groups.

3.3 Discussion

The present data demonstrate that, with the exception of BW723C86, all the 5-HT_2 receptor agonists tested, mCPP, WAY 161503, Ro 60-0175 and DOI when given i.v. caused activation of the external urethral sphincter (EUS) in urethane anaesthetized rats with a primed (i.e. 80% full) urinary bladder. In addition, with the exceptions of DOI and BW723C86, they all increased urethral pressure and inhibited the micturition reflex. In this latter respect DOI had an excitatory effect, while BW723C86 was ineffective on the micturition reflex. The three agonists mCPP (see Bonhaus *et al.*, 1997) WAY 161503 (Cryan & Lucki, 2000) and Ro-60-0175 (Martin *et al.*, 1998) are considered to be selective for 5-HT_{2C} receptors. If taken alone these agonist data would suggest that activation of 5-HT_{2C} receptors causes external urethral sphincter activation, urethral contraction and the inhibition of micturition. It should be noted that an increase in urethral pressure could involve both the urethral smooth muscle and striated muscle due to the structural link of both urethral muscle types. Further, the present data confirm the observations by Steers & De Groat (1989) that mCPP activates the external urethral sphincter. However, the suggestion by these authors that the inhibition of isovolumetric contractions by mCPP is caused by activation of the EUS is unfounded. The present data indicate that this is a separate action, as although mCPP completely inhibited the micturition reflex, the other agonists increased EUS-EMG in a similar fashion but only attenuated the micturition reflex.

To confirm whether the evoked responses caused by mCPP, WAY 161503 and Ro 60-0175 on the micturition variables involved activation of 5-HT_{2C} receptors; the effects of

these agonists was studied in the presence of the selective 5-HT_{2C} receptor antagonists. RS 102221 (Bonhaus *et al.*, 1997) failed to block the mCPP evoked responses on the EUS and the micturition reflex. In fact, the mCPP-evoked EUS-EMG activity tended to be potentiated. This may simply reflect the lack of selectivity of mCPP; for instance, mCPP is a partial agonist at 5-HT_{2A} receptors (Grotewiel *et al.*, 1994) as well as 'other' receptors. To confirm that these effects were in fact mediated by 5-HT_2 receptors another selective 5-HT_{2C} receptor agonist, WAY 161503, was tested against the non-selective 5-HT_2 receptor antagonist mianserin (see Bonhaus *et al.*, 1995). Both doses of mianserin blocked the ability of WAY 161503 to evoke EUS-EMG activity but mianserin had little effect on the inhibitory action of WAY 161503 on the micturition reflex. However, the high dose of mianserin did block the ability of WAY 161503 to increase residual volume and to significantly increase urethral pressure. Thus these data are at least consistent with the view that external urethral sphincter activation involves 5-HT_2 receptors. To further clarify if the 5-HT_{2C} receptor subtype was responsible for this action of WAY 161503 experiments were carried out again in the presence of the RS 102221. Once more the same dose (500 $\mu g\ kg^{-1}$) that had failed to block the effects of mCPP also failed to affect the effects of WAY 161503 on EUS-EMG and urethral pressure, although it blocked all the inhibitory actions of WAY 161503 on the micturition reflex. Further, this high dose of RS 102221, as for mCPP, tended to potentiate the effects of WAY 161503 on the EUS-EMG. Nevertheless, the lowest dose did block WAY 161503-evoked EUS-EMG activity but not the increase in urethral pressure or the inhibitory action on the micturition reflex. This would indicate that the increase in EUS activity by these agonists is separate from the ability of these drugs to inhibit micturition. The failure of the high doses to

affect the evoked EUS-EMG is difficult to explain, although RS 102221 was reported to have inconsistent actions on 5-HT_{2C} receptor mediated effects (Bonhaus *et al.*, 1997). For instance, RS 102221 failed to block the classical $5HT_{2C}$ receptor mediated effect of hypolocomotion (Bonhaus *et al.*, 1997) evoked by mCPP at any dose. Thus the present data extend and confirm that the use of RS 102221, at least *in vivo,* to characterise 5-HT_{2C} receptor mediated effects needs to be dealt with caution as the combined data suggest RS 102221 is having other unknown actions. However, overall the present data are still consistent with the view that 5-HT_{2C} receptors are involved in EUS-EMG activation and have an inhibitory action on the micturition reflex. This was further confirmed by studying the effects of Ro 60-0175, which is considered to have at least 500x greater selectivity for 5-HT_{2C} over 5-HT_{2A} and 5-HT_{2B} receptors (see Barnes & Sharp 1999), in the presence of the highly selective 5-HT_{2C} receptor antagonist SB 242084 (Kennett *et al.*, 1997). It should be noted however that *in vitro* studies have recently revealed Ro 60-0175 possesses greater selectivity for both human and rat 5-HT_{2B} receptors than previously thought (Ki = 2.4 nM; Kimura *et al.*, 2004) therefore caution needs to be applied again when characterising 5-HT_{2C} receptor mediated effects. The Ro 60-0175 evoked EUS-EMG and inhibitory effects on the micturition reflex were now blocked but not the increase in urethral pressure, implying that the evoked increase in urethral pressure is most likely to be mediated by another 5-HT_2 receptor subtype, possibly 5-HT_{2B} receptors. However, in this respect it is surprising that the 5-HT_{2B} receptor agonist BW723C86 (Kennett *et al.*, 1996) did not affect urethral pressure although there was tendency for it to increase this variable. This might again be related to lack of expected selectivity *in vivo*. Indeed *in vitro* data gives conflicting data on

BW723C86's selectivity for 5-HT_{2B} receptors, either 100x over 5-$HT_{2A/2C}$ (Porter *et al.*, 1999) or none (Knight *et al.*, 2004). Confirmation that 5-HT_{2B} receptors are responsible for the increase in urethral pressure by these agonists is the present observation that pre-treatment with the selective 5-HT_{2B} receptor antagonist RS 127445 (Bonhaus *et al.*, 1999) completely blocked the ability of Ro 60-0175 to increase urethral pressure without affecting the evoked increase in EUS-EMG and inhibition of the micturition reflex. This again indicates that increases in both urethral pressure and EUS activity are not linked.

The ability of DOI, which shows some selectivity for 5-HT_{2A} receptors (Porter *et al.*, 1999; Knight *et al.*, 2004), to increase EUS-EMG, implies that 5-HT_{2A} receptors are also involved in this action. DOI also has an excitatory action on the micturition reflex implying that this is a function of 5-HT_{2A} receptors. These conclusions are supported by the observations that DOI in the presence of the selective 5-HT_{2A} receptor antagonists, ketanserin (see Bonhaus *et al.*, 1997) or MDL 100907 (Kehne *et al.*, 1996) failed to evoke EUS-EMG activity and reversed the excitatory action of DOI on the micturition reflex to inhibition. This latter effect would be consistent with DOI, at the dose used in the present study, having an additional action on 5-HT_{2C} receptors. Thus the 5-HT_{2C} receptor mediated effect of DOI is overridden by its 5-HT_{2A} receptor agonist action on the micturition reflex. In this respect, DOI might be expected to also have a residual action on the external urethral sphincter. However, DOI only had a tendency to increase urethral pressure in the presence of these 5-HT_{2A} receptor antagonists, which is presumably due to a weak action on 5-HT_{2B} receptors as the selectivity of DOI between the 5-HT_2 receptor subtypes is very low (Porter *et al.*, 1999).

Overall the data suggest that both 5-HT_{2A} and 5-HT_{2C} receptors are involved in activation of the EUS. Thus to investigate if any 5-HT_{2A} receptor component was involved in the effects of WAY 161503 on the external urethral sphincter, studies were carried out in the presence of the 5-HT_{2A} receptor antagonists. Surprisingly the WAY 161503 evoked EUS-EMG was blocked by these antagonists but not the increase in urethral pressure. Further, the inhibitory action of WAY 161503 on the micturition reflex was unaffected, although the low dose of ketanserin tended to potentiate this action of WAY 161503 by probably blocking a weak excitatory 5-HT_{2A} receptor agonist action of WAY 161503. The failure of these antagonists to interfere with the WAY 161503 inhibitory action on the micturition reflex further confirms the conclusion that 5-HT_{2C} receptors play an inhibitory role in the control of micturition. Consistent with this conclusion is the observation that both ketanserin and MDL 100907 also failed to interfere with the inhibitory effects of Ro 60-0175 on the micturition reflex, although again blocking the ability of Ro 60-0175 to evoke EUS-EMG activity. Interestingly, although ketanserin did not interfere with the ability of Ro 60-0175 to evoke an increase in urethral pressure, MDL 100907 did prevent Ro 60-0175 evoking a significant increase in urethral pressure by increasing the variability of Ro 60-0175's action on this micturition variable. This latter observation implies that even at the low dose of MDL 100907 there might be some weak 5-HT_{2B} receptor actions. Thus overall the data indicate that both 5-HT_{2A} and 5-HT_{2C} receptors are involved in mediating the ability of these agonists to activate the external urethral sphincter. The relationship between these two 5-HT receptor subtypes remains to be determined in this action although on the micturition reflex they have an opposing action.

The present data examining the effects of ketanserin and MDL 100907 alone on the micturition reflex indicate that 5-HT_{2A} receptors may play a physiological tonic excitatory role in micturition as these antagonists caused increases in the volume threshold and ketanserin also caused an increase in the pressure threshold. However these antagonists had no effect on the reflex evoked EUS-EMG activity indicating that these receptors are not involved in this part of the micturition pathway. In this respect, ketanserin has previously been reported to inhibit bladder isovolumetric contractions (Testa *et al.*, 2001). However, the involvement of 5 HT_{2A} receptors in the reflex control of micturition may be considered a minor one as a similar dose to that of ketanserin of a 5-HT_7 receptor antagonist completely blocked the reflex (Read *et al.*, 2003). Further, Testa *et al.*, (2001) found that the selective 5-HT_{2C} receptor antagonist SB 242084 (Kennett *et al.*, 1997) and the so-called classical 5-HT_{2C} receptor antagonist mesulergine, which again according to binding data shows poor selectivity for this receptor (see Bonhaus *et al.*, 1997; Knight *et al.*, 2004) also failed to affect the micturition reflex (Testa *et al.*, 1999, 2001). The failure of the non-selective antagonist mianserin to affect the micturition reflex in the present study is somewhat surprising, however mianserin is known to bind to both α_1- and α_2-adrenoceptors, to histamine H_1 receptors and to 5-HT_{1D} receptors (see Marek *et al.*, 2003) and these receptor especially α-adrenoceptors are known to play a role in micturition (see Michel & Vrydag, 2006).

5-HT_2 receptors are also known to play a role in central and peripheral regulation of blood pressure. Activation of central 5-HT_{2A} receptors causes sympathoexcitation and vasopressin release (see Ramage, 2001) and, peripherally, vasoconstriction (see Kaumann

& Levy, 2006) all leading to a rise in blood pressure. The role of 5-HT_{2C} receptors in central cardiovascular regulation is poorly understood, although there is some evidence to suggest that they may cause sympathoexcitation and thus a rise in blood pressure (Knowles & Ramage, 2000). In the peripheral cardiovascular system 5-HT_{2C} receptors have not been found (see Kaumann & Levy, 2006). Consistent with this is that DOI, in the present experiments, caused an increase in blood pressure as did the so called selective 5-HT_{2C} receptor agonists, WAY 161503 and Ro 60-0175. However, the classical 5-HT_{2C} receptor agonist mCPP had no effect on blood pressure and neither did the selective 5-HT_{2B} receptor BW723C86. This latter observation is consistent with previous observations with BW723C86 (Knowles & Ramage, 2000), although high doses of BW723C86 given centrally caused a fall in blood pressure, which was believed to be mediated by 5-HT_{2B} receptors (Knowles & Ramage, 2000). The pressor action of WAY 161503 was observed to be attenuated by mianserin or RS 102221 at a high dose. These pre-treatments at least confirm that the action of WAY 161503 is at least mediated by 5-HT_2 receptors. Pre-treatment with the selective 5-HT_{2A} receptor antagonists, ketanserin at 100 but not 30 $\mu g\ kg^{-1}$ and MDL 100907 (30 $\mu g\ kg^{-1}$) attenuated the rise in blood pressure caused by WAY 161503, implying that the pressor effect caused by this agonist is mainly mediated by 5-HT_{2A} receptors. This would imply that RS 102221 at the high dose used in the present study is also blocking 5-HT_{2A} receptors, although why this antagonist at this high dose (500 $\mu g\ kg^{-1}$) fails to interfere with EUS-EMG evoked by WAY 161503 still remains unclear (see above). Interestingly, the same dose of MDL 100907 that attenuated the pressor effect of WAY 161503 failed to block the pressor effects of Ro 60-0175. However, this rise in blood pressure evoked by Ro 60-0175 could

be due to activation of central 5-HT_{2C} receptors and that Ro 60-0175 is more selective for 5-HT_{2C} over 5-HT_{2A} receptors. However, in the presence of the 5-HT_{2C} selective antagonist SB 242084 the pressor effect of Ro 60-0175 was potentiated. This may simply reflect the fact that Ro 60-0175 no longer evoked a bradycardia and thus there was no simultaneous reduction in cardiac output counteracting the evoked rise in blood pressure. This therefore still indicates that Ro 60-0175's pressor action is not mediated by 5-HT_{2C} receptors but by 5-HT_{2A} receptors. However, why would an effective 5-HT_{2A} receptor blocking dose of MDL 100907 fail to interfere with pressor response of Ro 60-0175? In fact MDL 100907 also tended to potentiate this response. This could be because MDL 100907 is interfering with 5-HT_{2B} receptors as indicated by a tendency for a reduction in the evoked increase in urethral pressure caused by Ro 60-0175 in the presence of MDL 100907 (see above). Thus the interference with the 5-HT_{2B} receptor depressor action would further explain the potentiation of the pressor response. In this respect Ro 60-0175 tended to have a larger pressor effect in the presence of the selective 5-HT_{2B} receptor antagonist but it was not significant. In addition, Ro 60-0175 at the dose chosen may have a more potent action than WAY 161503 at 5-HT_{2A} receptors and the dose of MDL 100907 was not enough to interfere with Ro 60-0175's pressor action. In this respect, the DOI evoked pressor action, which is considered to be mainly 5-HT_{2A} receptor mediated, was not attenuated by this low dose of MDL 100907. However, the rise in blood pressure could now be evoked by DOI acting on 5-HT_{2C} receptors. Further it might also be expected that the WAY 161503 pressor action would be potentiated by MDL 100907, as WAY-161503 caused an increase in urethral pressure, a putative 5-HT_{2B} receptor agonist mediated action. A possible explanation for not observing this for WAY 161503 as for

Ro 60-0175 is that Ro 60-0175 has a more potent agonist action on 5-HT_{2B} receptors and although the effects of Ro 60-0175 are larger in increasing urethral pressure (a putative 5-HT_{2B} receptor mediated action) it is not significant. Again the data are to a degree consistent with the view that activation of 5-HT_{2A} or 5-HT_{2C} receptors causes a rise while activation of 5-HT_{2B} receptors causes fall in blood pressure. In this respect, mCPP in the presence of RS-102221 (500 $\mu g\ kg^{-1}$) now caused a fall in blood pressure which would be at least consistent with a possible 5-HT_{2B} receptor action. However the surprising poor selectivity of these 5-HT_2 receptor ligands for the different 5-HT_2 receptor subtypes and the fact that all 3 subtypes are involved in cardiovascular regulation make it difficult to obtain a clear view of the function of these receptors in cardiovascular regulation.

In the case of a 5-HT_{2A} receptor agonist given intravenously the evoked increase in blood pressure observed could be due to vascular smooth contraction and/or central sympathoexcitation plus the release of vasopressin which also causes peripheral vasoconstriction (see Ramage, 2001). If the rise in blood pressure is due to a peripheral action; direct vasoconstriction and/or vasopressin release, it may be expected that a reflex baroreceptor mediated bradycardia to be associated with the evoked rise in blood pressure. However all 5-HT_2 receptor agonists tested in the present study, which evoked a rise in blood pressure, with the exception of mCPP, displayed no change in heart rate, which is a characteristic effect of 5-HT_2 receptor agonists (see Ramage, 2001). A further complicating effect to understanding the cardiovascular changes caused by 5-HT_{2A} receptor agonists is that DOI given i.v. or centrally to the hindbrain fails to cause sympathoexcitation of the sympathetic supply to the heart (Ramage & Daly, 1998),

although it activates all other vascular sympathetic outputs. Moreover the physiological role of these receptors in cardiovascular regulation is also poorly understood as the 5-HT_2 receptor antagonist had very little effect overall on resting blood pressure (see Ramage, 2001). In the present study, however the selective 5-HT_{2A} receptor antagonist MDL 100907 caused a significant increase in blood pressure and mianserin also tended to increase blood pressure although in all cases heart rate was unaffected, while ketanserin had no effect. This is somewhat surprising as blockade of 5-HT_2 receptor would be expected to cause a fall in blood pressure and this is the first report of a 5-HT_2 receptor antagonist causing an increase in blood pressure (see Ramage, 2001). The failure to see this trend with ketanserin may be related to its ability to block α_1-adrenoceptors (Ramage, 1985). A possible explanation for this increase in blood pressure could be related to blockade of 5-HT_{2B} receptors which could be under tonic activation; however the selective 5-HT_{2B} receptor antagonist RS 127445 given before agonist challenge did not affect resting blood pressure, so the mechanism by which MDL 100907 increases blood pressure may be unrelated. Thus, as with micturition all 5-HT_2 receptor subtypes seem to be involved in cardiovascular regulation and with the seeming lack of selectivity of these ligands and the fact that these receptors activate different parts of the cardiovascular system that have opposing/interacting actions makes it difficult again to clearly understand the precise function of the individual 5-HT_2 receptor subtypes in this system.

3.4 Conclusion

It can be concluded that 5-$HT_{2A/2C}$ receptors activate the external urethral sphincter (EUS); however there is no evidence that these receptors are involved in the reflex regulation of this muscle during micturition. In addition, activation of 5-HT_{2B} receptors can cause an increase in urethral pressure and this effect still occurs when the evoked EUS-EMG activity is blocked thus indicating that 5-HT_{2B} receptor are causing contraction of the urethral smooth muscle. Again in the present experiments urethral pressure was unaffected by the agonists whilst testing the micturition reflex thus the physiological role of these receptors cannot as yet be elucidated. Finally, 5-HT_{2C} receptor activation causes cause inhibition of the micturition reflex; again the physiological role is not clear, although hypothesised to be important in the 5-HT_{1A} receptor mediated pathway which is known to be involved in micturition (see Ramage, 2006). Additionally, data from chapter 4 infusion experiments would suggest that 5-HT_{2C} receptors are physiologically involved in inhibition of the micturition reflex. However there is some evidence in the present and other studies (see above) that 5-HT_{2A} receptors play a small excitatory role in the control of micturition. The physiological role of 5-HT_2 receptor subtypes in cardiovascular regulation is also unclear from the present experiments as it is from the literature (see Ramage 2001). However, activation of 5-$HT_{2A/2C}$ receptors causes a pressor response, while activation of 5-HT_{2B} receptors causes a depressor response. Another problematic aspect of studying the function of 5-HT_2 receptor subtypes in vivo is the seemingly poor selectivity of ligands used to study these receptors.

Experimental group	n	EUS-EMG (with bladder filled to 80% VT)				Urethral Pressure (UP)			Mean Arterial Pressure (MAP)			Heart Rate (HR)		
		Baseline μV	Test μV	Δ %	Onset s	Baseline mmHg	Test mmHg	Δ %	Baseline mmHg	Test mmHg	Δ	Baseline bpm	Test bpm	Δ
Saline + Saline	5	0.26 ± 0.02	0.30 ± 0.01	-	-	22 ± 2	21 ± 0.4	-	125 ± 3	127 ± 1	-	329 ± 12	338 ± 4	-
DMSO + Saline	5	0.26 ± 0.04	0.26 ± 0.02	-	-	22 ± 2	22 ± 2	-	125 ± 3	125 ± 3	-	329 ± 12	336 ± 2	-
300 μg kg^{-1} WAY 161503	5	0.26 ± 0.04	0.44 ± 0.02	179 ± 17	28 ± 11	22 ± 2	25 ± 1	13 ± 2	119 ± 8	127 ± 1	21 ± 1	336 ± 9	338 ± 4	1 ± 1
300 μg kg^{-1} Ro 60-0175	5	0.28 ± 0.03	0.74 ± 0.06	154 ± 15	36 ± 13	22 ± 2	27 ± 1	23 ± 2	116 ± 4	131 ± 2	14 ± 1	346 ± 10	335 ± 2	-13 ± 2
300 μg kg^{-1} mCPP	5	0.42 ± 0.10	1.00 ± 0.10	166 ± 17	20 ± 5	19 ± 2	23 ± 1	24 ± 3	140 ± 4	140 ± 3	0.1 ± 2	363 ± 11	342 ± 3	-21 ± 3
30 μg kg^{-1} DOI	3	0.40 ± 0.06	1.00 ± 0.10	139 ± 27	48 ± 9	24 ± 2	27 ± 1	11 ± 1	121 ± 5	134 ± 1	13 ± 1	369 ± 12	371 ± 2	4 ± 2
50 μg kg^{-1} DOI	3	0.37 ± 0.07	1.00 ± 0.10	224 ± 36	46 ± 6	21 ± 1	26 ± 4	5 ± 1	111 ± 10	120 ± 2	10 ± 1	377 ± 12	362 ± 4	-16 ± 3
100 μg kg^{-1} DOI	5	0.56 ± 0.12	2.00 ± 0.10	198 ± 15	17 ± 3	20 ± 2	21 ± 1	4 ± 2	121 ± 8	142 ± 3	21 ± 1	337 ± 30	341 ± 7	1 ± 2
300 μg kg^{-1} BW723C86	5	0.34 ± 0.02	0.41 ± 0.01	-	-	23 ± 3	24 ± 1	5 ± 1	116 ± 4	123 ± 1	6 ± 1	349 ± 9	335 ± 2	-12 ± 2

Table 3.4a 5-HT_2 receptor agonist i.v. experiments in urethane anaesthetized rats: baseline and test values and changes (Δ) in external urethral sphincter (EUS) EMG, urethral pressure (UP), mean blood pressure (MAP) and heart rate (HR)

Experimental Group		Volume threshold			Pressure Threshold			Residual Volume		
		Baseline ml	Test ml	Δ %	Baseline mmHg	Test mmHg	Δ %	Baseline %	Test %	Δ %
Saline + Saline	5	0.27 ± 0.01	0.26 ± 0.05	-	9 ± 0.5	8 ± 1	-	58 ± 10	55 ± 11	-
DMSO + Saline	5	0.42 ± 0.06	0.42 ± 0.06	-	9 ± 1	9 ± 1	-	43 ± 9	43 ± 9	-
300 μg kg^{-1} WAY 161503	5	0.33 ± 0.09	0.49 ± 0.12	55 ± 13	8 ± 0.5	10 ± 1	25 ± 11	31 ± 6	61 ± 4	121 ± 35
300 μg kg^{-1} Ro 60-0175	5	0.30 ± 0.06	0.48 ± 0.07	71 ± 15	10 ± 1	18 ± 3	90 ± 33	36 ± 9	71 ± 8	142 ± 61
300 μg kg^{-1} mCPP	5	0.29 ± 0.01	-	-	10 ± 1	-	-	43 ± 12	-	-
30 μg kg^{-1} DOI	3	0.38 ± 0.03	0.28 ± 0.02	-27 ± 5	10 ± 1	10 ± 1	-4 ± 6	38 ± 14	38 ± 14	8 ± 19
50 μg kg^{-1} DOI	3	0.31 ± 0.09	0.23 ± 0.08	-22 ± 17	11 ± 3	9 ± 2	-20 ± 4	53 ± 14	30 ± 7	-12 ± 8
100 μg kg^{-1} DOI	5	0.31 ± 0.06	0.22 ± 0.06	-35 ± 7	12 ± 1	9 ± 1	-19 ± 4	32 ± 8	31 ± 5	20 ± 28
300 μg kg^{-1} BW723C86	5	0.40 ± 0.06	0.49 ± 0.05	27 ± 13	9 ± 1	11 ± 1	30 ± 9	22 ± 7	35 ± 5	116 ± 65

Table 3.4b 5-HT_2 receptor agonist i.v. experiments in urethane anaesthetized rats: baseline and test values and changes (Δ) in volume threshold, pressure threshold and residual volume

Experimental group	n	EUS-EMG (with bladder filled to 80% VT)				Urethral Pressure (UP)			Mean Arterial Pressure (MAP)			Heart Rate (HR)		
		Baseline μV	Test μV	Δ %	Onset s	Baseline mmHg	Test mmHg	Δ %	Baseline mmHg	Test mmHg	Δ	Baseline bpm	Test bpm	Δ
300 μg kg^{-1} Mianserin	5	0.56 ± 0.02	0.54 ± 0.02	-	-	24 ± 4	25 ± 1	2 ± 1	112 ± 8	137 ± 2	24 ± 3	335 ± 10	347 ± 10	13 ± 2
30 μg kg^{-1} SB 242084	5	0.40 ± 0.05	0.43 ± 0.14	-	-	18 ± 1	17 ± 0.2	-0.4 ± 1	100 ± 5	109 ± 1	8 ± 1	347 ± 12	351 ± 3	4 ± 1
100 μg kg^{-1} Ketanserin	5	0.36 ± 0.07	0.36 ± 0.02	-	-	20 ± 2	21 ± 1	4 ± 1	110 ± 5	111 ± 0.2	1 ± 0.3	369 ± 6	366 ± 1	-3 ± 1
30 μg kg^{-1} MDL 100907	5	0.36 ± 0.06	0.41 ± 0.02	-	-	24 ± 1	25 ± 1	3 ± 0.5	115 ± 5	131 ± 1	14 ± 1	356 ± 14	344 ± 3	-12 ± 3

Table 3.5a 5-HT_2 receptor antagonist i.v. experiments in urethane anaesthetized rats: baseline and test values and changes (Δ) in external urethral sphincter (EUS) EMG, urethral pressure (UP), mean blood pressure (MAP) and heart rate (HR)

Experimental Group		Volume threshold			Pressure Threshold			Residual Volume		
		Baseline ml	Test ml	Δ %	Baseline mmHg	Test mmHg	Δ %	Baseline %	Test %	Δ %
300 μg kg^{-1} Mianserin	5	0.31 ± 0.07	0.37 ± 0.06	27 ± 17	10 ± 1	10 ± 2	11 ± 14	33 ± 1	45 ± 10	42 ± 32
30 μg kg^{-1} SB 242084	5	0.28 ± 0.05	0.32 ± 0.02	23 ± 12	9 ± 0.5	9 ± 1	5 ± 10	35 ± 6	31 ± 10	-10 ± 18
100 μg kg^{-1} Ketanserin	5	0.27 ± 0.02	0.34 ± 0.04	27 ± 4	11 ± 1	12 ± 1	11 ± 4	43 ± 4	61 ± 4	51 ± 23
30 μg kg^{-1} MDL 100907	5	0.25 ± 0.03	0.41 ± 0.06	65 ± 15	8 ± 1	11 ± 2	32 ± 24	30 ± 5	54 ± 11	89 ± 35

Table 3.5b 5-HT_2 receptor antagonist i.v. experiments in urethane anaesthetized rats: baseline and test values and changes (Δ) in volume threshold, pressure threshold and residual volume

Experimental group	n	EUS-EMG (with bladder filled to 80% VT)				Urethral Pressure (UP)			Mean Arterial Pressure (MAP)			Heart Rate (HR)		
		Baseline μV	Test μV	Δ %	Onset s	Baseline mmHg	Test mmHg	Δ %	Baseline mmHg	Test mmHg	Δ	Baseline bpm	Test bpm	Δ
100 μg kg^{-1} Mianserin + WAY	5	0.38 ± 0.06	0.40 ± 0.01	-	-	24 ± 1	28 ± 1	15 ± 2	122 ± 6	131 ± 2	8 ± 1	319 ± 26	330 ± 5	7 ± 1
300 μg kg^{-1} Mianserin + WAY	5	0.42 ± 0.12	0.46 ± 0.03	-	-	24 ± 4	25 ± 1	7 ± 2	121 ± 5	128 ± 2	7 ± 1	364 ± 11	344 ± 3	-17 ± 2
30 μg kg^{-1} RS 102221 + WAY	3	0.33 ± 0.03	0.43 ± 0.03	-	-	28 ± 0.5	31 ± 1	14 ± 2	105 ± 10	126 ± 1	21 ± 2	342 ± 29	330 ± 4	-17 ± 3
100 μg kg^{-1} RS 102221 + WAY	4	0.28 ± 0.03	0.91 ± 0.14	186 ± 30	23 ± 5	25 ± 3	27 ± 1	8 ± 1	115 ± 11	148 ± 16	18 ± 2	366 ± 13	376 ± 2	3 ± 1
500 μg kg^{-1} RS 102221 + WAY	5	0.28 ± 0.02	1.20 ± 0.10	299 ± 36	26 ± 5	20 ± 4	22 ± 1	11 ± 1	108 ± 3	116 ± 1	7 ± 1	375 ± 11	367 ± 3	-7 ± 1
500 μg kg^{-1} RS 102221 + mCPP	5	0.42 ± 0.06	2.00 ± 0.20	533 ± 84	11± 3	17 ± 2	19 ± 1	19 ± 3	133 ± 5	118 ± 2	-15 ± 2	364 ± 8	352 ± 3	-20 ± 4
30 μg kg^{-1} SB 242084 + Ro 60-0175	5	0.24 ± 0.11	0.29 ± 0.01	-	-	15 ± 2	20 ± 0.2	27 ± 3	107 ± 6	142 ± 1	31 ± 2	396 ± 29	380 ± 1	-12 ± 1

Table 3.6a 5-HT_{2C} receptor antagonists on agonist responses i.v. experiments in urethane anaesthetized rats: baseline and test values and changes (Δ) in external urethral sphincter (EUS) EMG, urethral pressure (UP), mean blood pressure (MAP) and heart rate (HR)

Experimental Group		Volume threshold			Pressure Threshold			Residual Volume		
		Baseline ml	Test ml	Δ %	Baseline mmHg	Test mmHg	Δ %	Baseline %	Test %	Δ %
100 μg kg^{-1} Mianserin + WAY	5	0.41 ± 0.06	0.45 ± 0.06	25 ± 10	9 ± 1	10 ± 1	11 ± 14	44 ± 4	57 ± 4	38 ± 20
300 μg kg^{-1} Mianserin + WAY	5	0.32 ± 0.05	0.37 ± 0.08	19 ± 13	11 ± 1	13 ± 1	21 ± 11	44 ± 10	50 ± 11	12 ± 23
30 μg kg^{-1} RS 102221 + WAY	3	0.38 ± 0.13	0.70 ± 0.21	90 ± 15	9 ± 1	7 ± 2	54 ± 8	33 ± 12	64 ± 7	125 ± 48
100 μg kg^{-1} RS 102221 + WAY	4	0.24 ± 0.1	0.38 ± 0.1	56 ± 12	10 ± 2	14 ± 3	40 ± 5	48 ± 6	58 ± 9	13 ± 4
500 μg kg^{-1} RS 102221 + WAY	5	0.22 ± 0.05	0.23 ± 0.05	4 ± 12	8 ± 0.5	8 ± 1	4 ± 13	43 ± 9	32 ± 5	-14 ± 23
500 μg kg^{-1} RS 102221 + mCPP	5	0.30 ± 0.02	-	-	8 ± 0.5	-	-	35 ± 10	-	-
30 μg kg^{-1} SB 242084 + Ro 60-0175	5	0.30 ± 0.03	0.20 ± 0.02	-53 ± 16	8 ± 1	10 ± 1	10 ± 9	37 ± 5	90 ± 40	39 ± 15

Table 3.6b 5-HT_{2C} receptor antagonists on agonist responses i.v. experiments in urethane anaesthetized rats: baseline and test values and changes (Δ) in volume threshold, pressure threshold and residual volume

Experimental group	n	EUS-EMG (with bladder filled to 80% VT)				Urethral Pressure (UP)			Mean Arterial Pressure (MAP)			Heart Rate (HR)		
		Baseline μV	Test μV	Δ %	Onset s	Baseline mmHg	Test mmHg	Δ %	Baseline mmHg	Test mmHg	Δ	Baseline bpm	Test bpm	Δ
30 μg kg^{-1} Ketanserin + WAY	5	0.48 ± 0.1	0.46 ± 0.02	-	-	21 ± 3	23 ± 1	9 ± 1	124 ± 11	150 ± 2	25 ± 3	369 ± 3	345 ± 3	-20 ± 3
100 μg kg^{-1} Ketanserin + WAY	5	0.38 ± 0.06	0.43 ± 0.01	-	-	17 ± 4	19 ± 1	14 ± 1	120 ± 9	124 ± 3	5 ± 1	344 ± 8	334 ± 2	-9 ± 1
30 μg kg^{-1} MDL 100907 + WAY	5	0.58 ± 0.08	0.58 ± 0.03	-	-	27 ± 3	29 ± 1	7 ± 1	138 ± 9	144 ± 2	4 ± 1	365 ± 10	340 ± 3	-22 ± 3
30 μg kg^{-1} Ketanserin + Ro 60-0175	5	0.22 ± 0.01	0.30 ± 0.03	-	-	20 ± 0.1	22 ± 0.3	9 ± 1	95 ± 0.3	111 ± 2	16 ± 2	390 ± 15	356 ± 3	-34 ± 2
30 μg kg^{-1} MDL 100907 + Ro 60-0175	5	0.22 ± 0.02	0.40 ± 0.01	-	-	15 ± 0.1	17 ± 0.2	11 ± 2	102 ± 2	121 ± 2	18 ± 2	369 ± 18	344 ± 3	-22 ± 3
30 μg kg^{-1} Ketanserin + DOI	5	0.34 ± 0.06	0.40 ± 0.02	-	-	19 ± 1	21 ± 1	11 ± 2	137 ± 9	151 ± 1	14 ± 1	335 ± 10	346 ± 3	8 ± 2
30 μg kg^{-1} MDL 100907 + DOI	3	0.40 ± 0.06	0.47 ± 0.03	-	-	25 ± 0.5	27 ± 0.3	7 ± 1	112 ± 4	139 ± 1	24 ± 3	360 ± 1	363 ± 2	2 ± 1

Table 3.7a 5-HT_{2A} receptor antagonists on agonist responses i.v. experiments in urethane anaesthetized rats: baseline and test values and changes (Δ) in external urethral sphincter (EUS) EMG, urethral pressure (UP), mean blood pressure (MAP) and heart rate (HR)

Experimental Group		Volume threshold			Pressure Threshold			Residual Volume		
		Baseline ml	Test ml	Δ %	Baseline mmHg	Test mmHg	Δ %	Baseline %	Test %	Δ %
30 μg kg^{-1} Ketanserin + WAY	5	0.41 ± 0.1	1 ± 0.33	153 ± 33	8 ± 1	13 ± 2	61 ± 17	36 ± 11	55 ± 11	104 ± 81
100 μg kg^{-1} Ketanserin + WAY	5	0.29 ± 0.1	0.51 ± 0.10	81 ± 13	10 ± 1	15 ± 2	53 ± 14	42 ± 6	74 ± 6	92 ± 18
30 μg kg^{-1} MDL 100907 + WAY	5	0.25 ± 0.04	0.50 ± 0.13	92 ± 23	8 ± 1	15 ± 2	83 ± 24	31 ± 5	58 ± 8	166 ± 120
30 μg kg^{-1} Ketanserin + Ro 60-0175	5	0.22 ± 0.02	0.58 ± 0.05	61 ± 5	8 ± 1	17 ± 1	50 ± 3	53 ± 7	64 ± 7	12 ± 17
30 μg kg^{-1} MDL 100907 + Ro 60-0175	5	0.34 ± 0.11	0.83 ± 0.17	63 ± 6	8 ± 1	14 ± 1	44 ± 6	61 ± 9	68 ± 13	-19 ± 41
30 μg kg^{-1} Ketanserin + DOI	5	0.37 ± 0.06	0.54 ± 0.12	43 ± 15	8 ± 0.5	14 ± 3	63 ± 22	31 ± 7	61 ± 5	135 ± 49
30 μg kg^{-1} MDL 100907 + DOI	3	0.32 ± 0.10	0.54 ± 0.1	90 ± 43	10 ± 1	18 ± 6	82 ± 63	51 ± 8	74 ± 4	49 ± 19

Table 3.7b 5-HT_{2A} receptor antagonists on agonist responses i.v. experiments in urethane anaesthetized rats: baseline and test values and changes (Δ) in volume threshold, pressure threshold and residual volume

Experimental group	n	EUS-EMG (with bladder filled to 80% VT)				Urethral Pressure (UP)			Mean Arterial Pressure (MAP)			Heart Rate (HR)		
		Baseline μV	Test μV	Δ %	Onset s	Baseline mmHg	Test mmHg	Δ %	Baseline mmHg	Test mmHg	Δ	Baseline bpm	Test bpm	Δ
30 μg kg^{-1} RS 127445 + Ro 60-0175	5	0.46 ± 0.02	1.40 ± 0.1	218 ± 30	19 ± 2	12 ± 2	10 ± 0.2	-11 ± 4	122 ± 5	152 ± 1	25 ± 2	356 ± 5	333 ± 8	-19 ± 6

Table 3.8a 5-HT_{2B} receptor antagonist on agonist responses i.v. experiments in urethane anaesthetized rats: baseline and test values and changes (Δ) in external urethral sphincter (EUS) EMG, urethral pressure (UP), mean blood pressure (MAP) and heart rate (HR)

Experimental Group		Volume threshold			Pressure Threshold			Residual Volume		
		Baseline ml	Test ml	Δ %	Baseline mmHg	Test mmHg	Δ %	Baseline %	Test %	Δ %
30 μg kg^{-1} RS 127445 + Ro 60-0175	5	0.40 ± 0.04	0.66 ± 0.05	39 ± 6	11 ± 1	21 ± 5	43 ± 7	27 ± 9	72 ± 4	64 ± 11

Table 3.8b 5-HT_{2B} receptor antagonist on agonist responses i.v. experiments in urethane anaesthetized rats: baseline and test values and changes (Δ) in volume threshold, pressure threshold and residual volume

Chapter 4

5-HT_2 receptor agonists and antagonists (infused i.v.) and their effects on the urethra and the micturition reflex

4.1 Introduction

In the previous chapter, it was established that all three 5-HT_2 receptor subtypes are involved in the control of bladder and urethral function. Experiments revealed that 5-$HT_{2A/2C}$ receptors caused activation of the external urethral sphincter, whilst 5-HT_{2B} receptors increased urethral pressure, probably by contracting urethral smooth muscles. Additionally, on the micturition reflex, 5-HT_{2C} receptors were observed to be inhibitory whereas 5-HT_{2A} receptors were excitatory. The experiments carried out in chapter 3 also revealed poor selectivity of the agonists for 5-HT_2 receptors especially when trying to distinguish between the 5-HT_{2A} and 5-HT_{2C} receptor subtype.

By utilizing an intravenous infusion dosing regimen, experiments carried out in this chapter made it possible to obtain free plasma concentrations of the compounds that would be selective for a particular 5-HT_2 receptor subtype. Ro 60-0175 was chosen as the compound of choice in this study as it is known as the most selective commercially available 5-HT_{2C} receptor agonist. Therefore carrying out initial pharmacokinetic and pharmacodynamic studies of Ro 60-0175 enabled accurate calculations that would be adequate for the compound to act selectively on 5-HT_{2C} receptors only. Discussions with Pfizer colleagues as well as data collected in-house indicated that free plasma concentrations of Ro 60-0175 at 10 nM would be selective for the 5-HT_{2C} receptor (see Methods) whereas the other two higher concentrations (30 and 100 nM) chosen would activate both 5-HT_{2C} and 5-HT_{2A} receptors. Furthermore, data indicated (see Methods) that targeting 3 nM free plasma concentration for the 5-HT_{2C} receptor antagonist SB 242084 and 5-HT_{2A} receptor antagonist MDL 100907 would be selective for their

respective receptor subtypes. Therefore, by targeting the above free plasma concentrations, it would be possible to determine if both receptors 5-HT_{2A} and 5-HT_{2C} or just one was involved in the activation of the external urethral sphincter. Performing this selectivity study also provided further evidence for an inhibitory and excitatory role for 5-HT_{2C} and 5-HT_{2A} receptors on the micturition reflex respectively. Experiments in this chapter were also carried out to further investigate the urethral pressure increases observed in chapter 3, as well as the involvement of 5-HT_2 receptors in cardiovascular regulation.

4.2 Results

Table 4.1 summarises the measured free plasma concentrations of 5-HT_{2C} receptor agonist Ro 60-0175, 5-HT_{2C} receptor antagonist SB 242084 and 5-HT_{2A} receptor antagonist MDL 100907 administered as infusion doses i.v., compared to simulated target concentrations in anaesthetised female rats.

Baseline values for all variables are shown in Tables 4.4-4.9.

4.2.1 Vehicle control

Intravenous infusion of saline (0.9% wv^{-1}, n = 5) or 4% CTE vehicle for 5-HT_2 receptor antagonists (See Drugs and Solutions section 2.6) (n = 5) evoked no significant changes in baseline EUS-EMG signal and urethral pressure or volume threshold, pressure threshold and residual volume caused by infusion of saline at a rate of 0.1 ml min^{-1} (Figure 4.1a & 4.1b). Baseline MAP and HR were also unaffected.

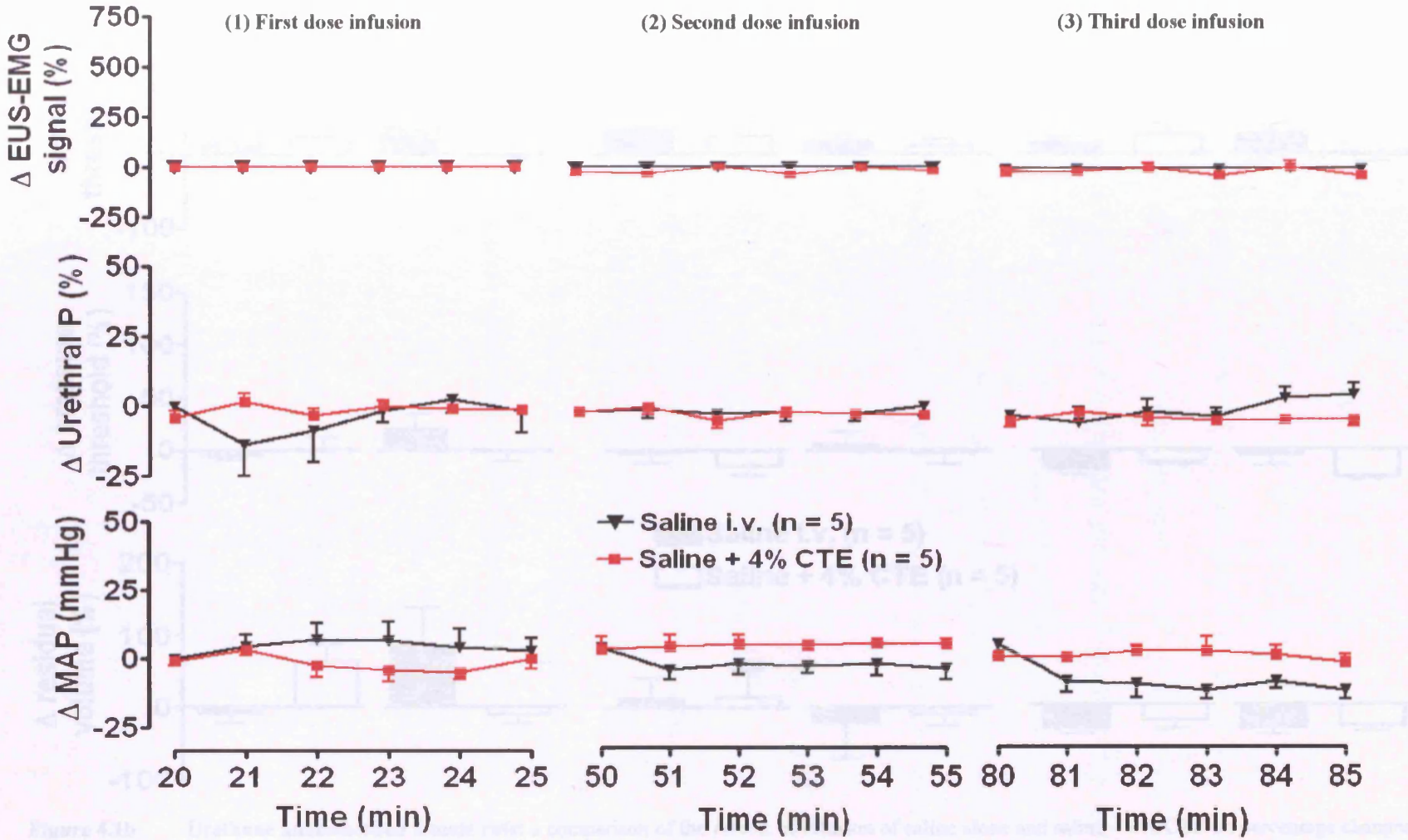

Figure 4.1a **Urethane anaesthetised female rats:** a comparison between the effects of infusion of saline alone and saline + 4% CTE on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (UP) and mean arterial blood pressure (MAP). Baseline variables were recorded for a duration of 5 min whilst the bladder was filled to 80% volume of saline required to elicit a micturition reflex. Panel (1) shows baseline variables recorded at 20-25 min after start of infusion of both saline alone and saline + 4% CTE, Panel (2) shows variables at 50-55 min after start of infusion of the two vehicles and Panel (3) shows variables at 80-85 min after start of infusion of the two vehicles. Each point represents the mean value and vertical bars show the s.e.mean.

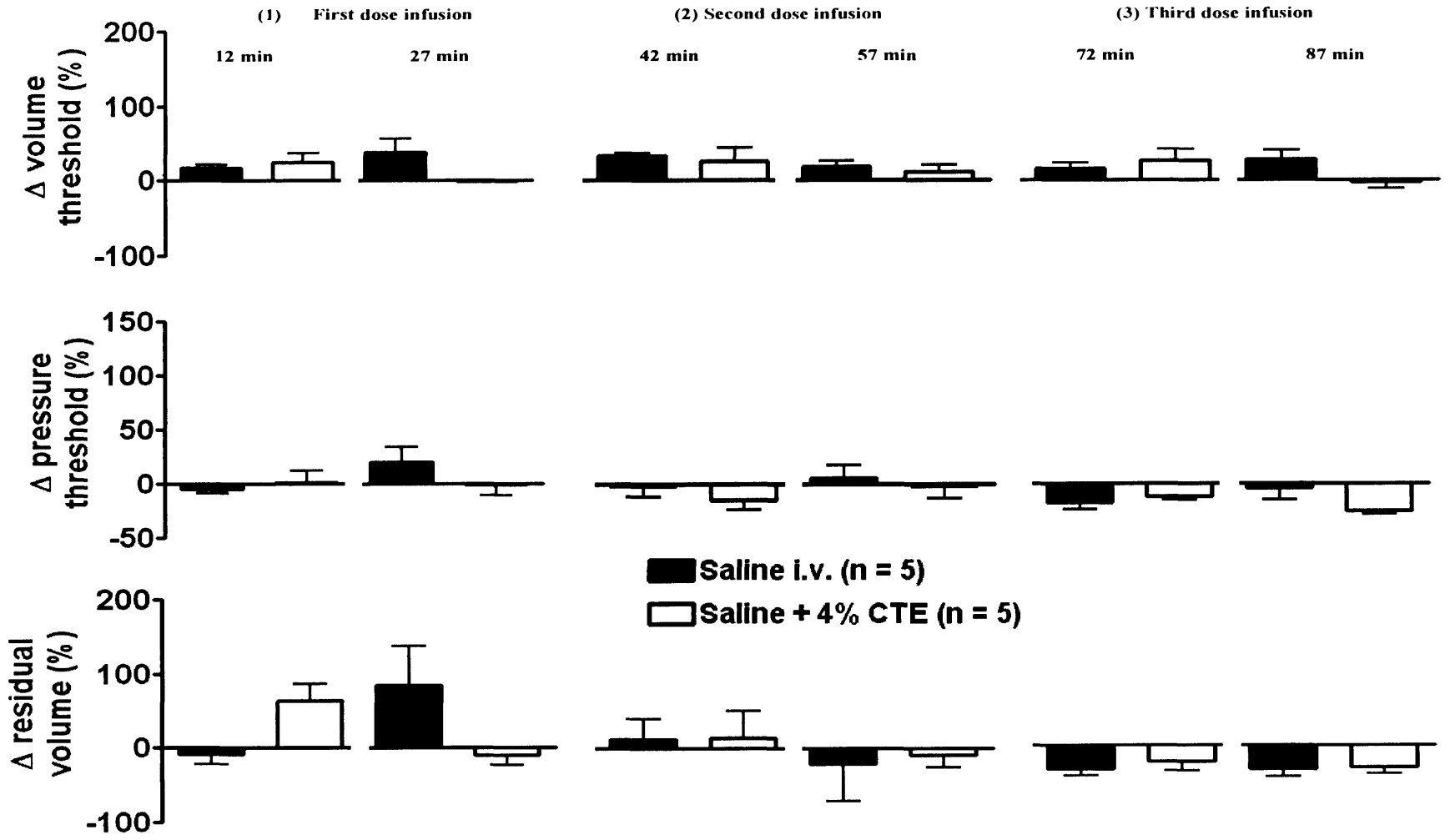

Figure 4.1b **Urethane anaesthetised female rats:** a comparison of the effects of infusion of saline alone and saline + 4% CTE on percentage changes (Δ) in volume threshold, pressure threshold and residual volume. Two micturition reflexes were tested during each infusion dose (~ 30 min for each infusion dose). Panel (1) shows variables recorded following testing the micturition reflex after 12 and 27 min infusions of both saline alone and saline + 4% CTE, Panel (2) shows variables recorded following testing the micturition reflex after 42 and 57 min infusions of the two vehicles and Panel (3) shows variables recorded following testing the micturition reflex after 72 and 87 min infusions of the two vehicles. Each bar represents the mean value and vertical bars show the s.e.mean.

4.2.2 5-HT_{2C} receptor agonist

4.2.2.1 I.v. infusion of Ro 60-0175 in saline

Traces showing the effects of Ro 60-0175 (27, 90 and 270 μg kg^{-1} min^{-1}; i.v.) on changes in EUS-EMG signal, urethral pressure and the micturition reflex are shown in Figure 4.2a. Infusion of Ro 60-0175 (27 and 90 μg kg^{-1} min^{-1}; n = 6) evoked no significant changes in baseline EUS-EMG signal and urethral pressure. Infusion of the high dose of Ro 60-0175 (270 μg kg^{-1} min^{-1}, i.v; n = 5) evoked EUS-EMG activity and significantly increased the EUS-EMG signal by 144 ± 11%. The onset of appearance of EUS-EMG activity following infusion of the high dose of Ro 60-0175 was 92 ± 25s. Mean urethral pressure was unaffected (Figure 4.2b).

On the micturition reflex, Ro 60-0175 (27 μg kg^{-1} min^{-1}) was inhibitory with a significant increase observed on volume (68 ± 10%; reflex 1) and pressure threshold (21 ± 8%; reflex 1; Figure 4.2c). In contrast, administration of both 90 μg kg^{-1} min^{-1} (-24 ± 14%; reflex 2) and 270 μg kg^{-1} min^{-1} (-51 ± 11% and -45 ± 12%; reflex 1 & 2) Ro 60-0175 were observed to be excitatory on the micturition reflex with significant decreases on volume threshold (Figure 4.2c).

None of the three doses of Ro 60-0175 had any significant effect on baseline MAP and HR.

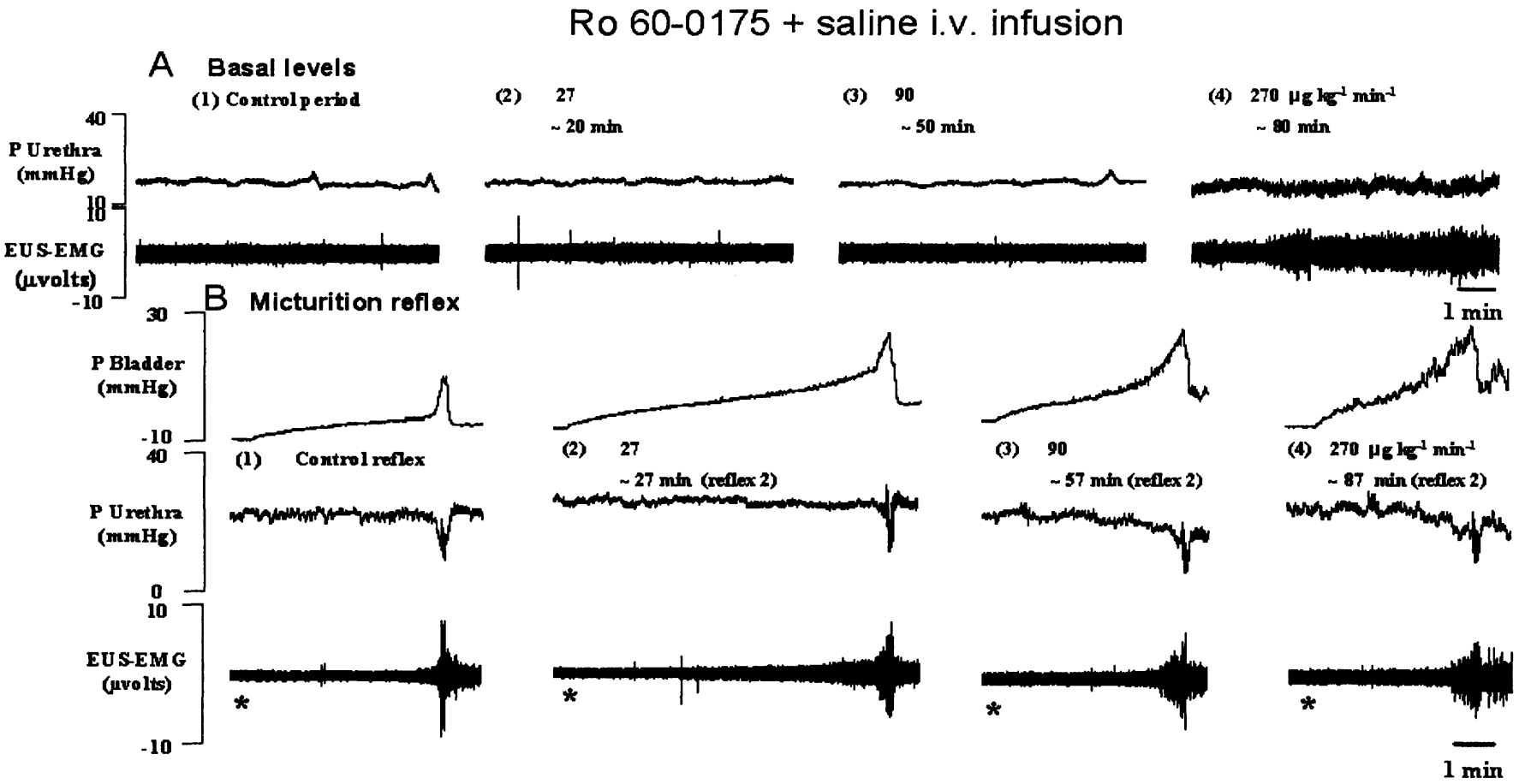

Figure 4.2a **Urethane anaesthetized female rat:** Traces showing the effects of simultaneous infusions (i.v.) of Ro 60-0175 in saline on **A** baseline variables; urethral pressure and EUS-EMG signal (with the bladder filled to 80% volume of saline required to elicit a micturition reflex) and **B** bladder distension-evoked micturition reflex showing bladder pressure (P Bladder), urethral pressure (P Urethra) and EUS-EMG. **A,** Baseline variables:- Panel (1) shows baseline variables during control period, Panel (2) shows variables at 20-25 min after start of infusion of Ro 60-0175 (27 µg kg^{-1} min^{-1}) and saline, Panel (3) shows variables at 50-55 min after start of infusion of both first dose Ro 60-0175 and saline and Panel (4) shows variables at 80-85 min after start of infusion of both first dose of Ro 60-0175 and saline. **B,** Micturition reflex:- Panel (1) – control reflex tested prior to start of infusion of Ro 60-0175 and saline, Panel (2) - reflex tested at 27 min after start of infusion of Ro 60-0175 and saline, Panel (3) - reflex tested at 57 min after start of infusion of Ro 60-0175 and saline and Panel (4) - reflex tested at 87 min after start of infusion of Ro 60-0175 and saline. * denotes onset of saline infusion into the bladder.
(The large signal recorded for P Urethra in **A** Panel 4 was biological rather than signal noise).

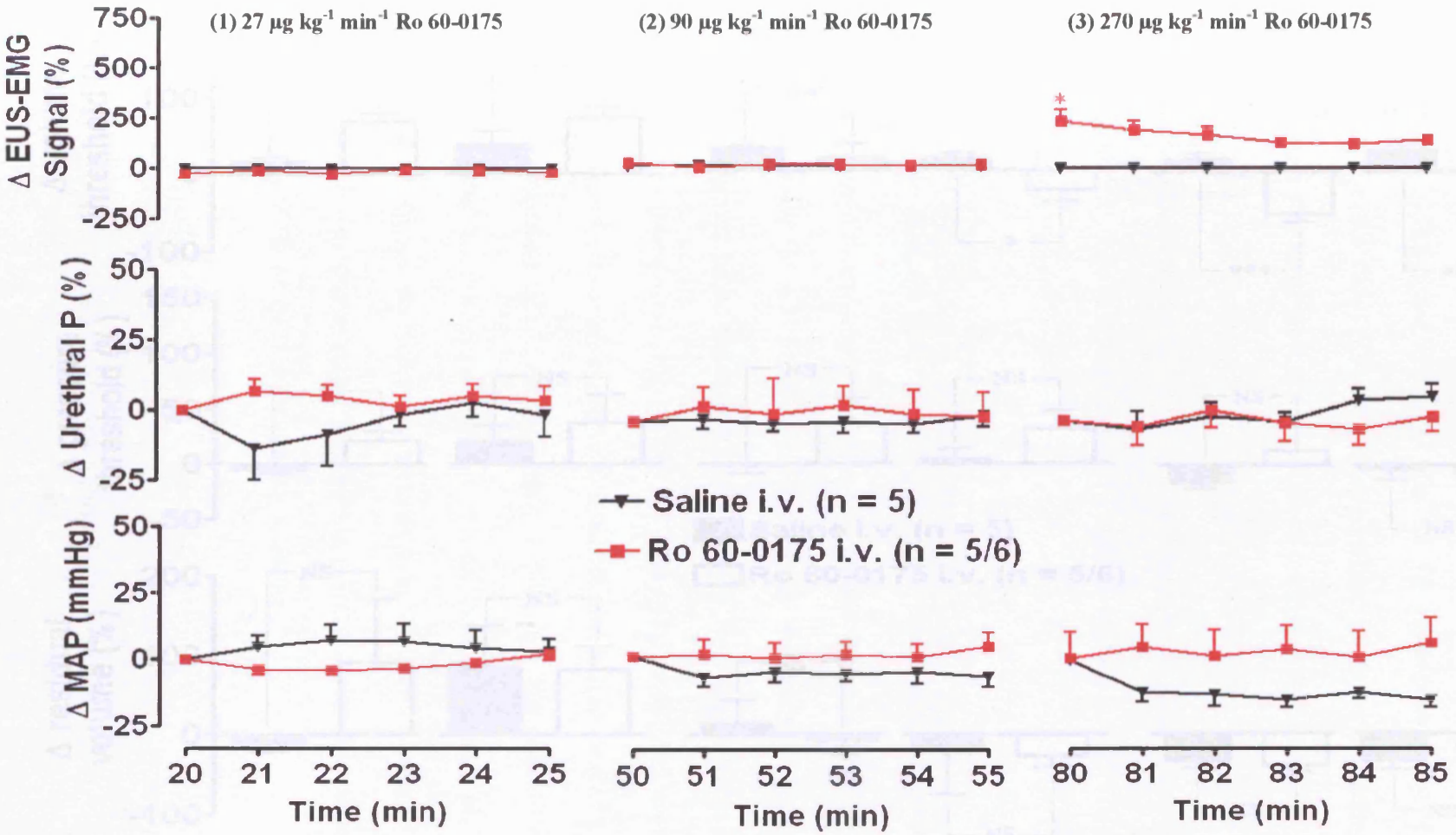

Figure 4.2b **Urethane anaesthetised female rats:** a comparison of the effects of infusion of Ro 60-0175 and vehicle (saline) on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (UP) and mean arterial blood pressure (MAP). Baseline variables were recorded for a duration of 5 min whilst the bladder was filled to 80% volume of saline required to elicit a micturition reflex. Panel (1) shows baseline variables recorded at 20-25 min after start of infusion of both Ro 60-0175 (27 µg kg^{-1} min^{-1}) and saline, Panel (2) shows variables at 50-55 min after start of infusion of first dose of Ro 60-0175 and saline and Panel (3) shows variables at 80-85 min after start of infusion of first dose of Ro 60-0175 and saline. Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by Ro 60-0175 were compared with saline control using two-way analysis of variance followed by the least significant difference test. * $P<0.05$.

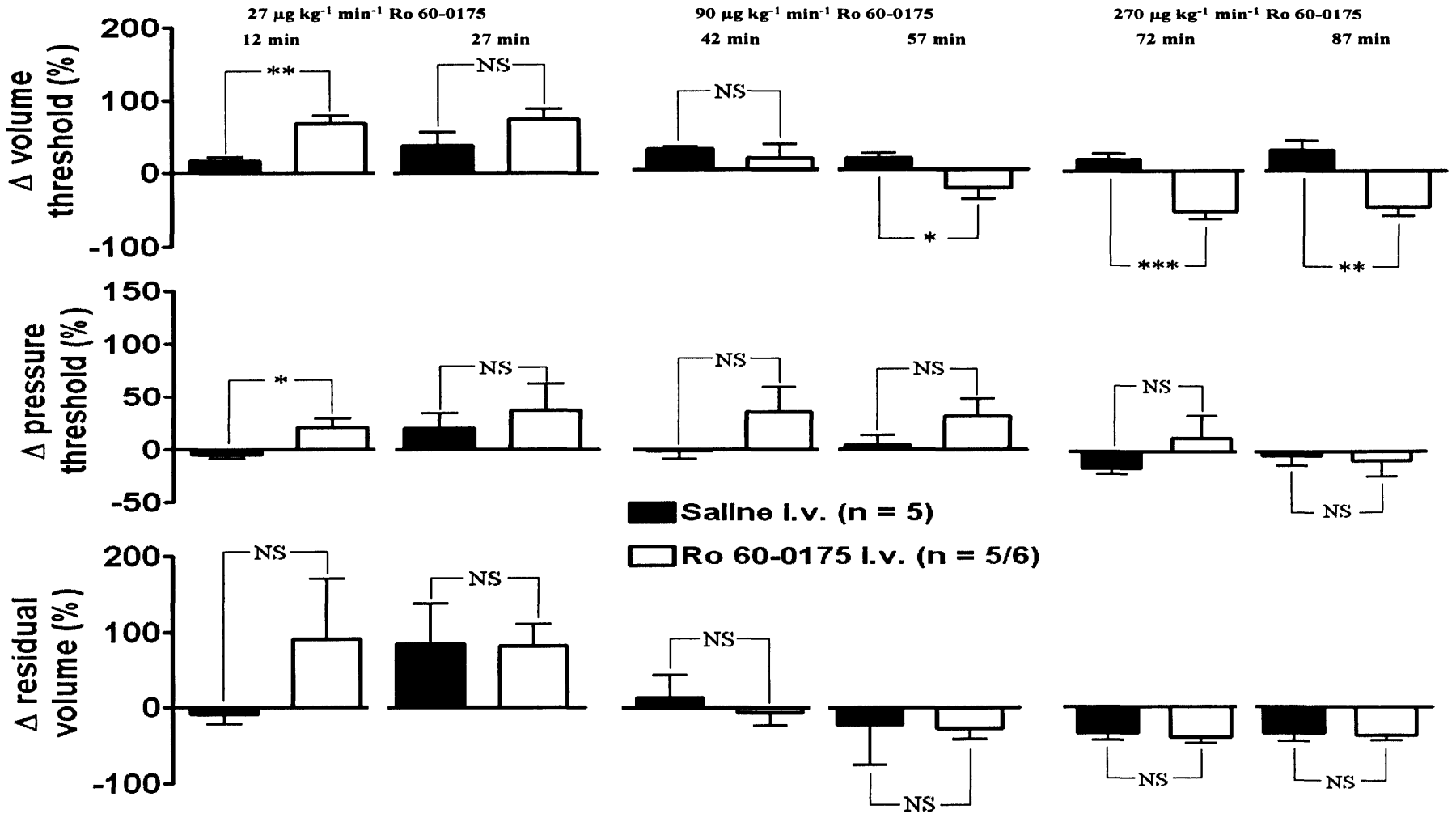

Figure 4.2c **Urethane anaesthetised female rats:** a comparison of the effects of infusion of vehicle (saline) and Ro 60-0175 on percentage changes (Δ) in volume threshold, pressure threshold and residual volume. Two micturition reflexes were tested during each infusion dose (~ 30 min duration for each infusion dose). Panel (1) shows variables recorded following testing the micturition reflex after 12 and 27 min infusions of both saline and Ro 60-0175 (27 µg kg^{-1} min^{-1}), Panel (2) shows variables recorded following testing the micturition reflex after 42 and 57 min infusions of saline and Ro 60-0175 (90 µg kg^{-1} min^{-1}) and Panel (3) shows variables recorded following testing the micturition reflex after 72 and 87 min infusions of saline and Ro 60-0175 (270 µg kg^{-1} min^{-1}). Each bar represents the mean value and vertical bars show the s.e.mean. * P<0.05, ** P<0.01, *** P<0.001, NS non-significant.

4.2.2.2 4% CTE and Ro 60-0175

Infusion of Ro 60-0175 (27 and 90 μg kg^{-1} min^{-1}; n = 5) in the presence of vehicle for 5-HT_2 receptor antagonists (4% CTE) evoked no significant changes in baseline EUS-EMG signal and urethral pressure. However, infusion of the high dose of Ro 60-0175 (270 μg kg^{-1} min^{-1}, n = 5) in the presence of 4% CTE evoked EUS-EMG activity and significantly increased the EUS-EMG signal by 1093 ± 141% (Figure 4.3b). The onset of appearance of EUS-EMG activity following infusion of the high dose of Ro 60-0175 in the presence of 4% CTE was 83 ± 14s.

On the micturition reflex, Ro 60-0175 (27 μg kg^{-1} min^{-1}) in the presence of 4% CTE was inhibitory, with a significant increase observed on volume threshold (59 ± 8%; reflex 2) and pressure threshold (55 ± 19%; reflex 1; Figure 4.3c). Interestingly, a significant increase was also observed on residual volume (105 ± 27%; reflex 2) at this dose. Unlike Ro 60-0175 in the presence of saline, Ro 60-0175 (90 μg kg^{-1} min^{-1}) in the presence of 4% CTE had no significant effect on volume threshold, but instead evoked a significant increase in pressure threshold (33 ± 15%; reflex 1). The high dose of Ro 60-0175 (270 μg kg^{-1} min^{-1}) caused a significant decrease in volume threshold (-43 ± 9 and -50 ± 10%; reflex 1 & 2) and a significant increase in pressure threshold (46 ± 22 and 53 ± 22%; reflex 1 & 2) which was interesting as the effect on pressure threshold was not observed following infusion of Ro 60-0175 with saline (see above).

Only the high dose of Ro 60-0175 (270 μg kg^{-1} min^{-1}) with 4% CTE caused a significant increase in MAP by 19 ± 1 mmHg (Figure 4.3b). HR was unaffected.

4.2.3 Effect of 5-HT_2 receptor antagonists on agonist responses

4.2.3.1 SB 242084 (5-HT_{2C}) and Ro 60-0175 (5-HT_{2C})

Traces showing the effects of Ro 60-0175 (270 μg kg^{-1} min^{-1}; i.v.) plus SB 242084 (22.5; 3.5 μg kg^{-1} min^{-1}; i.v.) on changes in EUS-EMG signal, urethral pressure and the micturition reflex are shown in Figure 4.3a. Ro 60-0175 (27 and 90 μg kg^{-1} min^{-1}; n = 3/5) in the presence of SB 242084 (22.5; 3.5 and 67.5; 3.5 μg kg^{-1} min^{-1}; n = 3/5) evoked no significant changes in baseline EUS-EMG signal and urethral pressure. Further, both doses of SB 242084 failed to block the highest infusion dose of Ro 60-0175 (270 μg kg^{-1} min^{-1}) evoked increase in baseline EUS-EMG signal (1381 ± 129% and 1259 ± 88%; Figure 4.3b). The onset of appearance of EUS-EMG activity following infusion of both doses of SB 242084 and the high dose of Ro 60-0175 was 100 ± 27 (low dose) and 113 ± 16 s (high dose) respectively. Urethral pressure was unaffected (Figure 4.3b).

The low dose of SB 242084 (22.5; 3.5 μg kg^{-1} min^{-1}) blocked the inhibitory effect of the lowest infusion dose of Ro 60-0175 (27 μg kg^{-1} min^{-1}) on the micturition reflex. Ro 60-0175 now had an excitatory effect on the micturition reflex causing a significant decrease in volume threshold (-29 ± 6%; reflex 2) and residual volume (-28 ± 12%; reflex 2). This excitatory effect on the micturition reflex was also observed at 90 and 270 μg kg^{-1} min^{-1} of Ro 60-0175 in combination with the low dose of SB 242084 resulting in decreases in volume threshold similar to those seen with Ro 60-0175 plus CTE vehicle (Figure 4.3c). Similarly, the high dose of SB 242084 (67.5; 3.5 μg kg^{-1} min^{-1}) significantly decreased the Ro 60-0175 (27 μg kg^{-1} min^{-1}) evoked increase in volume threshold (-16 ± 5%; reflex

1), and now resulted in a significant decrease in pressure threshold (-19 ± 6%; reflex 1) and residual volume (-36 ± 19 and -45 ± 23%; reflex 1 & 2; Figure 4.3c). Interestingly, although the middle infusion dose of Ro 60-0175 (90 μg kg^{-1} min^{-1}) alone had no effect on volume threshold and residual volume, in the presence of SB 242084 (22.5; 3.5 μg kg^{-1} min^{-1}), there were now excitatory actions observed, with decreases in volume threshold (-40 ± 6 and -44 ± 6%; reflex 1 & 2) and residual volume (-46 ± 14 and -48 ± 13%; reflex 1 & 2). Surprisingly, the low dose of SB 242084 failed to affect the increase in pressure threshold (21 ± 11 and 24 ± 4%; reflex 1 & 2). Again, with the high dose of SB 242084 (67.5; 3.5 μg kg^{-1} min^{-1}) and the middle infusion dose of Ro 60-0175 (90 μg kg^{-1} min^{-1}), volume threshold (-42 ± 7 and -40 ± 5%; reflex 1 & 2) and residual volume (-62 ± 6%; reflex 1) were reduced. A similar effect was also observed in the presence of the low dose of SB 242084 (22.5; 3.5 μg kg^{-1} min^{-1}) and the highest dose of Ro 60-0175 (270 μg kg^{-1} min^{-1}) where volume threshold (-46 ± 10 and -47 ± 10%; reflex 1 & 2) was observed to be decreased. Again, this dose of SB 242084 failed to affect the increase in pressure threshold (57 ± 13 and 65 ± 15%; reflex 1 & 2). Similarly, the high dose of SB 242084 (67.5; 3.5 μg kg^{-1} min^{-1}) failed to block the excitatory effect of Ro 60-0175 (270 μg kg^{-1} min^{-1}) on volume threshold (-55 ± 5 and -60 ± 3%; reflex 1 & 2). Although markedly reduced when compared to Ro 60-0175 alone, SB 242084 (67.5; 3.5 μg kg^{-1} min^{-1}) again failed to block the increase in pressure threshold in response to the high dose Ro 60-0175 on pressure threshold (7 ± 11%; reflex 2; see figure 4.3c for graphs).

Both doses of SB 242084 failed to block the pressor effects of Ro 60-0175 (270 μg kg^{-1} min^{-1}) on MAP (20 ± 0.3 (low dose) and 20 ± 1 mmHg (high dose) respectively; Figure 4.3b). HR was unaffected.

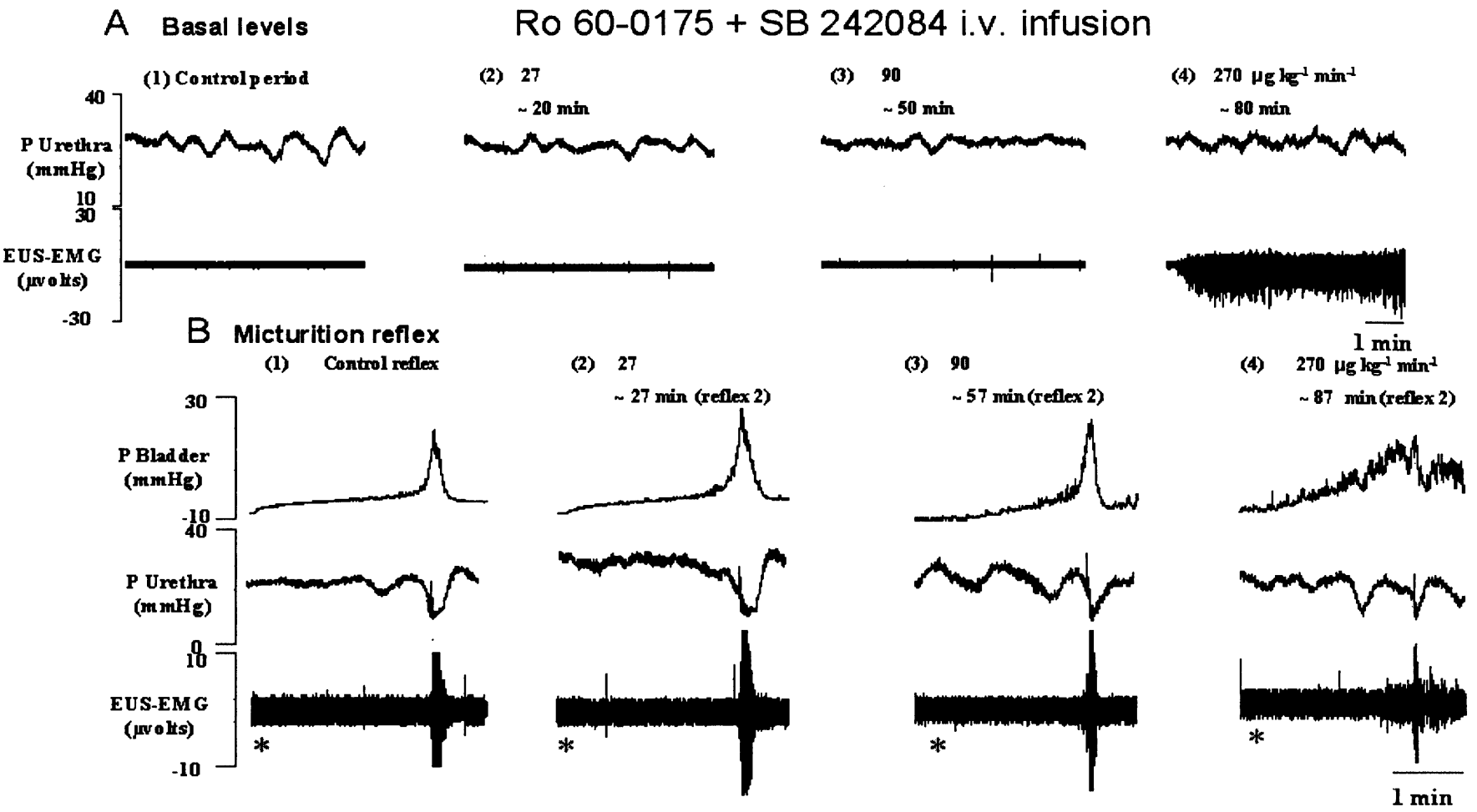

Figure 4.3a **Urethane anaesthetized female rat:** Traces showing the effects of simultaneous infusions (i.v.) of Ro 60-0175 and SB 242084 (22.5; 3.5 µg kg^{-1} min^{-1}) on **A** baseline variables; urethral pressure and EUS-EMG signal (with the bladder filled to 80% volume of saline required to elicit a micturition reflex) and **B** bladder distension-evoked micturition reflex showing bladder pressure (P Bladder), urethral pressure (P Urethra) and EUS-EMG. **A**, Baseline variables:- Panel (1) shows baseline variables during control period, Panel (2) shows these at 20-25 min after start of infusion of both Ro 60-0175 (27 µg kg^{-1} min^{-1}) and SB 242084, Panel 3 shows these at 50-55 min after start of infusion of first dose Ro 60-0175 and SB 242084 and Panel (4) shows these at 80-85 min after start of infusion of first dose of Ro 60-0175 and SB 242084. **B,** Micturition reflex:- Panel (1) – control reflex tested prior to start of infusion of Ro 60-0175 and SB 242084, Panel (2) – reflex tested 27 min after start of infusion of Ro 60-0175 and SB 242084, Panel (3) – reflex tested 57 min after start of infusion of Ro 60-0175 and SB 242084 and Panel (4) – reflex tested 87 min after start of infusion of Ro 60-0175 and SB 242084. * denotes onset of saline infusion into the bladder

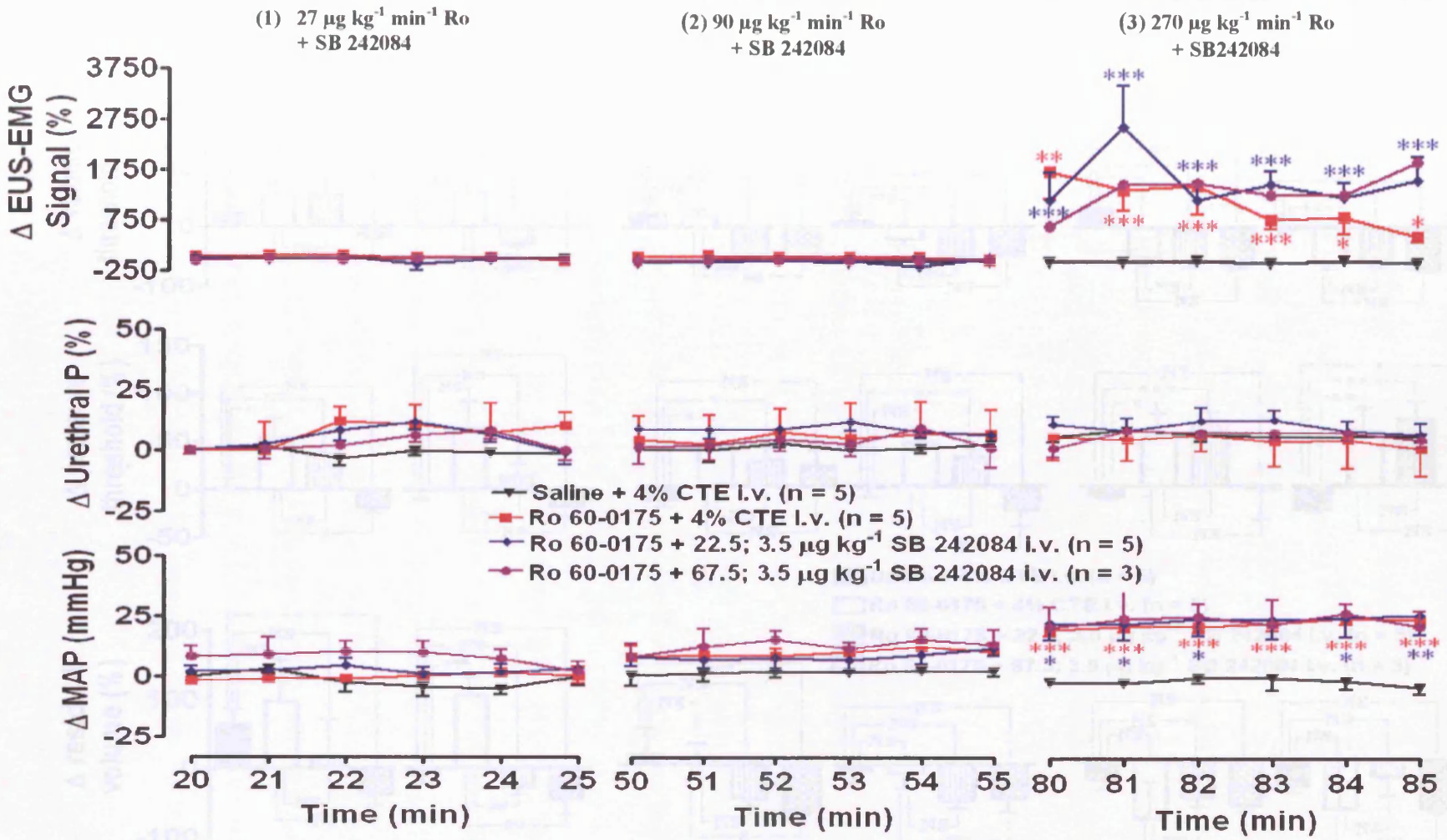

Figure 4.3b **Urethane anaesthetised female rats:** a comparison of the effects of infusion of Ro 60-0175 and SB 242084 on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (UP) and mean arterial blood pressure (MAP). Baseline variables were recorded for a duration of 5 min whilst the bladder was filled to 80% volume of saline required to elicit a micturition reflex. Panel (1) shows baseline variables recorded at 20-25 min after start of infusion of both Ro 60-0175 (27 µg kg^{-1} min^{-1}) and SB 242084 (22.5; 3.5 & 67.5; 3.5 µg kg^{-1} min^{-1}), Panel (2) shows variables at 50-55 min after start of infusion of first dose of Ro 60-0175 and SB 242084, and Panel (3) shows variables at 80-85 min after start of infusion of first dose of Ro 60-0175 and SB 242084. Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by Ro 60-0175 and SB 242084 were compared with vehicle using two-way analysis of variance followed by the least significant difference test. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

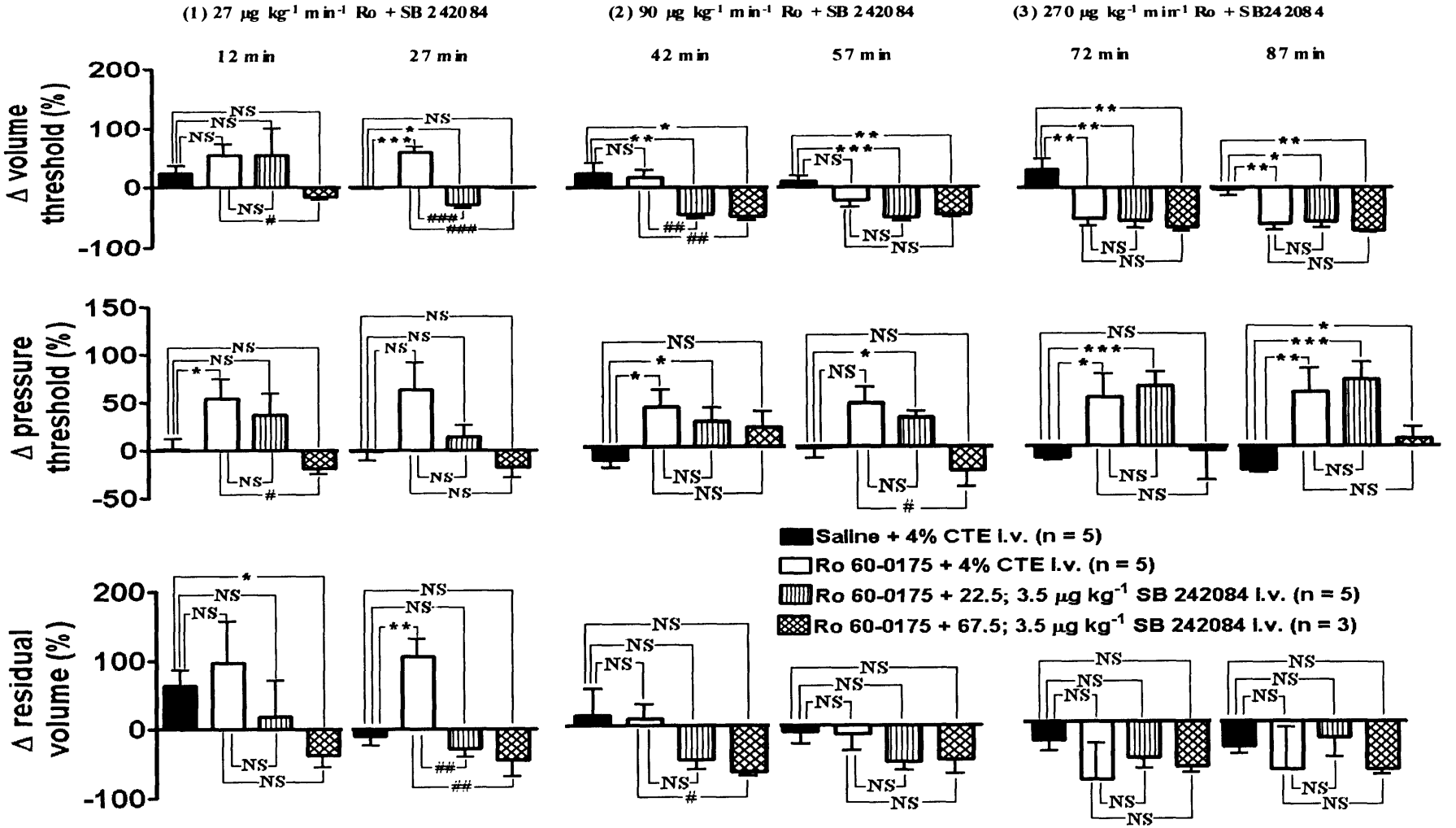

Figure 4.3c **Urethane anaesthetised female rats:** a comparison of the effects of infusion of Ro 60-0175 and SB 242084 on percentage changes (Δ) in volume threshold, pressure threshold and residual volume. Two micturition reflexes were tested during each infusion dose (~ 30 min duration for each infusion dose). Panel (1) shows variables recorded from testing the micturition reflex at 12 and 27 min after start of infusion of both Ro 60-0175 (27 μg kg^{-1} min^{-1}) and SB 242084 (22.5; 3.5 and 67.5; 3.5 μg kg^{-1} min^{-1}), Panel (2) shows variables recorded from testing the micturition reflex at 42 and 57 min after start of infusion of first dose of Ro 60-0175 and SB 242084 and Panel (3) shows variables recorded from testing the micturition reflex at 72 and 87 min after start of infusion of first dose of Ro 60-0175 and SB 242084. Each bar represents the mean value and vertical bars show the s.e.mean. Changes caused by Ro 60-0175 and SB 242084 were compared with vehicle using Student's unpaired t-test. *,# $P<0.05$, **,## $P<0.01$, ***, ### $P<0.001$, NS non-significant.

4.2.3.2 MDL 100907 ($5\text{-}HT_{2A}$) and Ro 60-0175 ($5\text{-}HT_{2C}$)

Traces showing the effects of Ro 60-0175 (27, 90 and 270 μg kg^{-1} min^{-1}; i.v.) plus MDL 100907 (16.7; 0.83 μg kg^{-1} min^{-1}; i.v.) on changes in EUS-EMG signal, urethral pressure and the micturition reflex are shown in Figure 4.4a. Ro 60-0175 (27 and 90 μg kg^{-1} min^{-1}; n = 5) in the presence of MDL 100907 (n = 5) evoked no significant changes in baseline EUS-EMG signal and urethral pressure. MDL 100907 significantly blocked the effects of high dose of Ro 60-0175 (270 μg kg^{-1} min^{-1}) on baseline EUS-EMG signal (Figure 4.4b).

On the micturition reflex, MDL 100907 failed to block the Ro 60-0175 (27 μg kg^{-1} min^{-1}) evoked increase in volume and pressure threshold and residual volume. Although the middle infusion dose of Ro 60-0175 (90 μg kg^{-1} min^{-1}) had no significant effect on volume threshold and residual volume, a combination of Ro 60-0175 and MDL 100907 was now inhibitory with significant increases observed on volume threshold (97 ± 28%; reflex 2) and residual volume (62 ± 13%; reflex 2). MDL 100907 failed to affect the Ro 60-0175 (90μg kg^{-1} min^{-1}) evoked increase in pressure threshold (93 ± 30 and 70 ± 20%; reflex 1 & 2). MDL 100907 significantly blocked the excitatory effect of the highest dose of Ro 60-0175 (270μg kg^{-1} min^{-1}) on volume threshold (83 ± 29 and 72 ± 22%; reflex 1 & 2), but again failed to block the inhibitory effect of Ro 60-0175 on pressure threshold (42 ± 12 and 43 ± 14%; reflex 1 & 2). Interestingly, although Ro 60-0175 (270 μg kg^{-1} min^{-1}) alone had no significant effect on residual volume, the combination of Ro 60-0175 and MDL 100907 caused a significant increase in residual volume by 103 ± 42% (reflex 1; see figure 4.4c for graphs).

A combination of MDL 100907 and Ro 60-0175 at 27 or 90 μg kg^{-1} min^{-1} now caused a significant decrease in MAP by -16 ± 0.4 and -16 ± 1 mmHg respectively, whilst MDL 100907 significantly attenuated the pressor effects of the highest dose infusion of Ro 60-0175 (270 μg kg^{-1} min^{-1}) on MAP (-17 ± 0.3 mmHg; Figure 4.4b). HR was again unaffected.

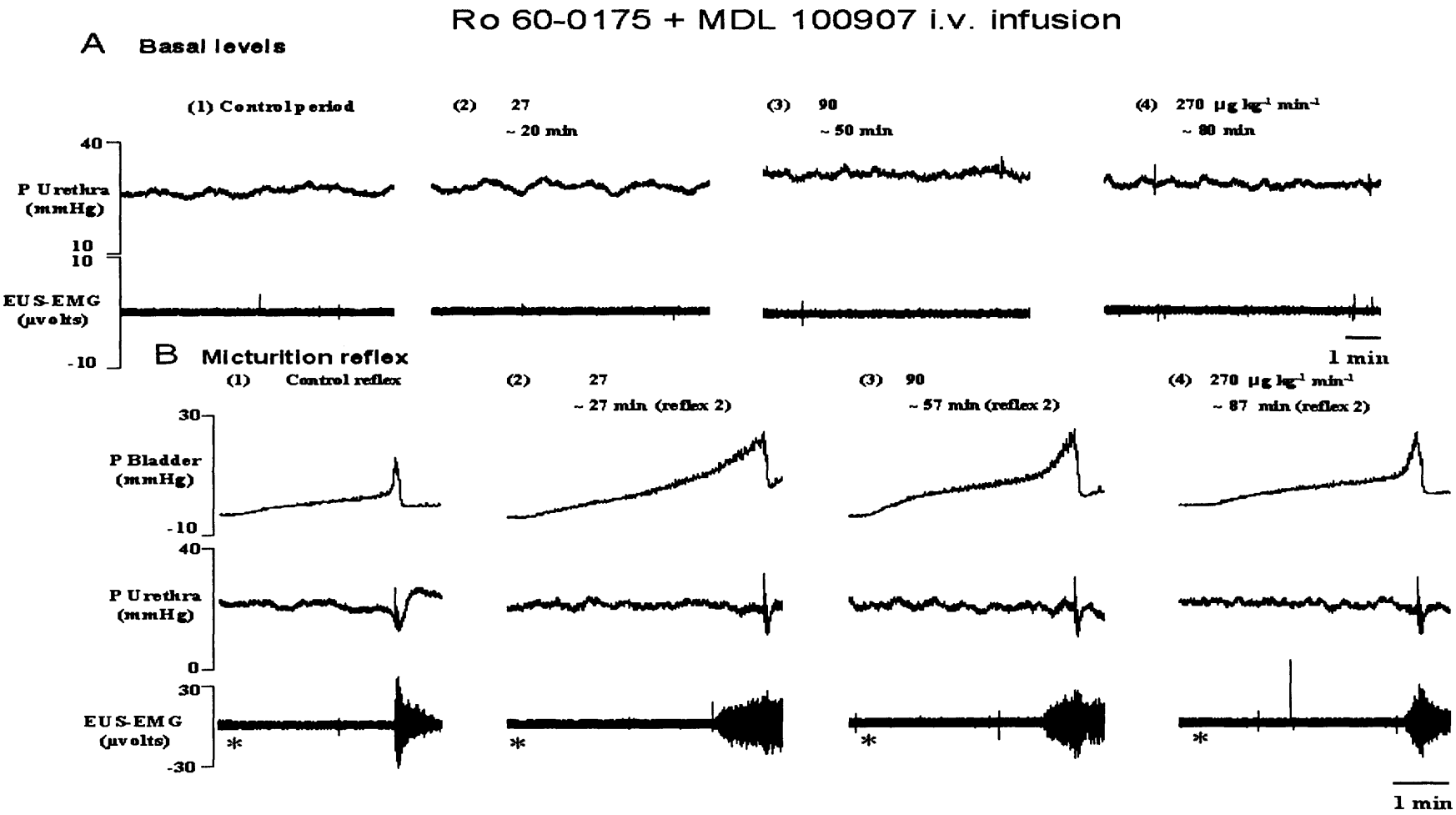

Figure 4.4a **Urethane anaesthetized female rat:** Traces showing the effects of simultaneous infusions (i.v.) of Ro 60-0175 and MDL 100907 (16.7; 0.83 µg kg^{-1} min^{-1}) on **A** baseline variables; urethral pressure and EUS-EMG signal (with the bladder filled to 80% volume of saline required to elicit a micturition reflex) and **B** bladder distension-evoked micturition reflex showing bladder pressure (P Bladder), urethral pressure (P Urethra) and EUS-EMG. **A**, Baseline variables:- Panel (1) shows baseline variables during control period, Panel (2) shows these at 20-25 min after start of infusion of both Ro 60-0175 (27 µg kg^{-1} min^{-1}) and MDL 100907, Panel 3 shows these at 50-55 min after start of infusion of both Ro 60-0175 and MDL 100907 and Panel (4) shows these at 80-85 min after start of infusion of both Ro 60-0175 and MDL 100907. **B**, Micturition reflex:- Panel (1) – control reflex tested prior to start of infusion of Ro 60-0175 and MDL 100907, Panel (2) – reflex tested 27 min after start of infusion of Ro 60-0175 and MDL 100907, Panel (3) – reflex tested 57 min after start of infusion of Ro 60-0175 and MDL 100907 and Panel (4) – reflex tested 87 min after start of infusion of Ro 60-0175 and MDL 100907.
* denotes onset of saline infusion into the bladder.

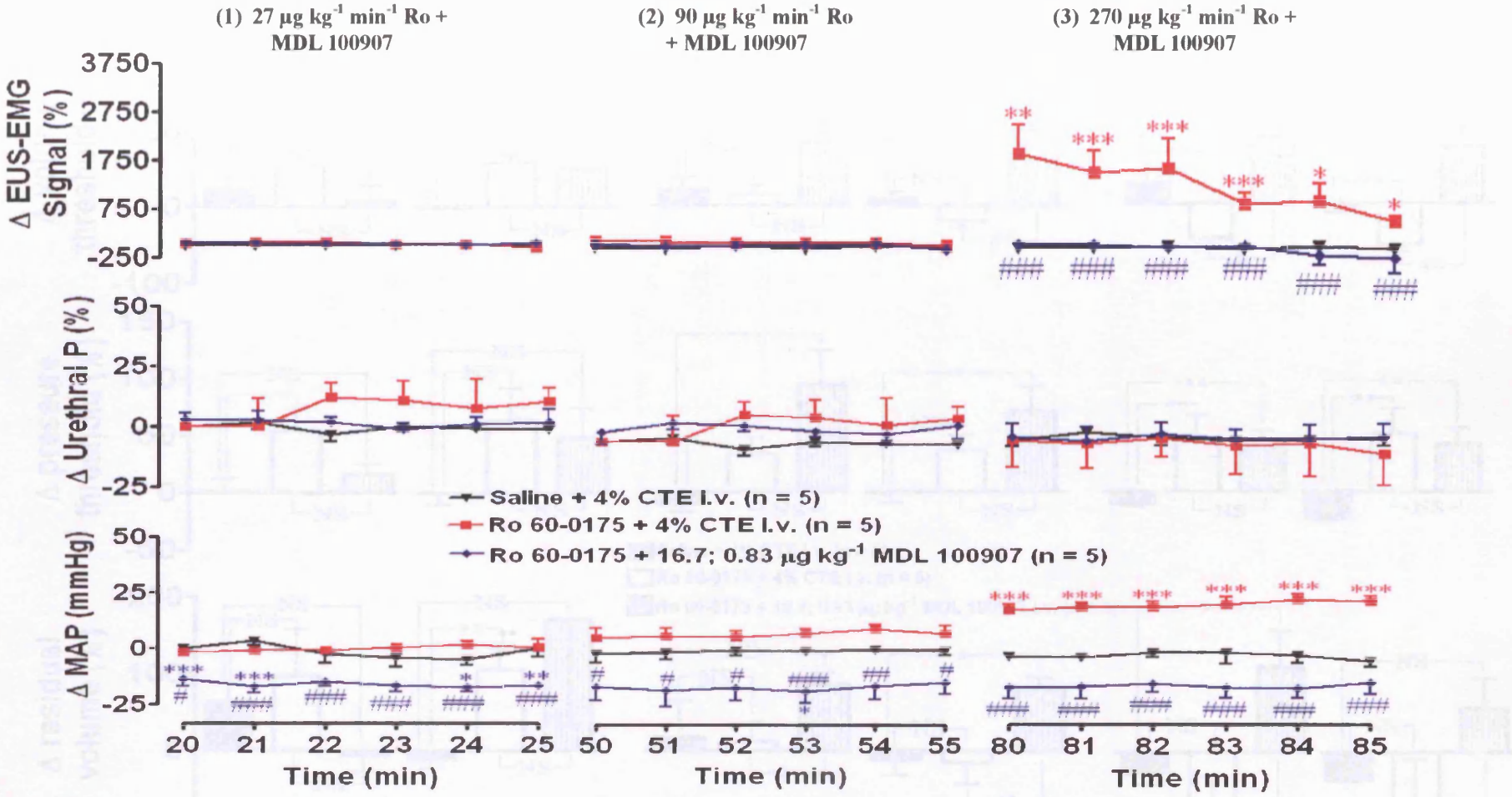

Figure 4.4b **Urethane anaesthetised female rats:** a comparison of the effects of infusion of Ro 60-0175 and MDL 100907 (16.7; 0.83 µg kg^{-1} min^{-1}) on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (UP) and mean arterial blood pressure (MAP). Baseline variables were recorded for a duration of 5 min whilst the bladder was filled to 80% volume of saline required to elicit a micturition reflex. Panel (1) shows baseline variables recorded at 20-25 min after start of infusion of both Ro 60-0175 (27 µg kg^{-1} min^{-1}) and MDL 100907, Panel (2) shows variables at 50-55 min after start of infusion of first dose Ro 60-0175 and MDL 100907, and Panel (3) shows variables at 80-85 min after start of infusion of first dose Ro 60-0175 and MDL 100907. Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by Ro 60-0175 and MDL 100907 were compared with vehicle using two-way analysis of variance followed by the least significant difference test. *, # P<0.05, **, ## P<0.01, ***, ### P<0.001.

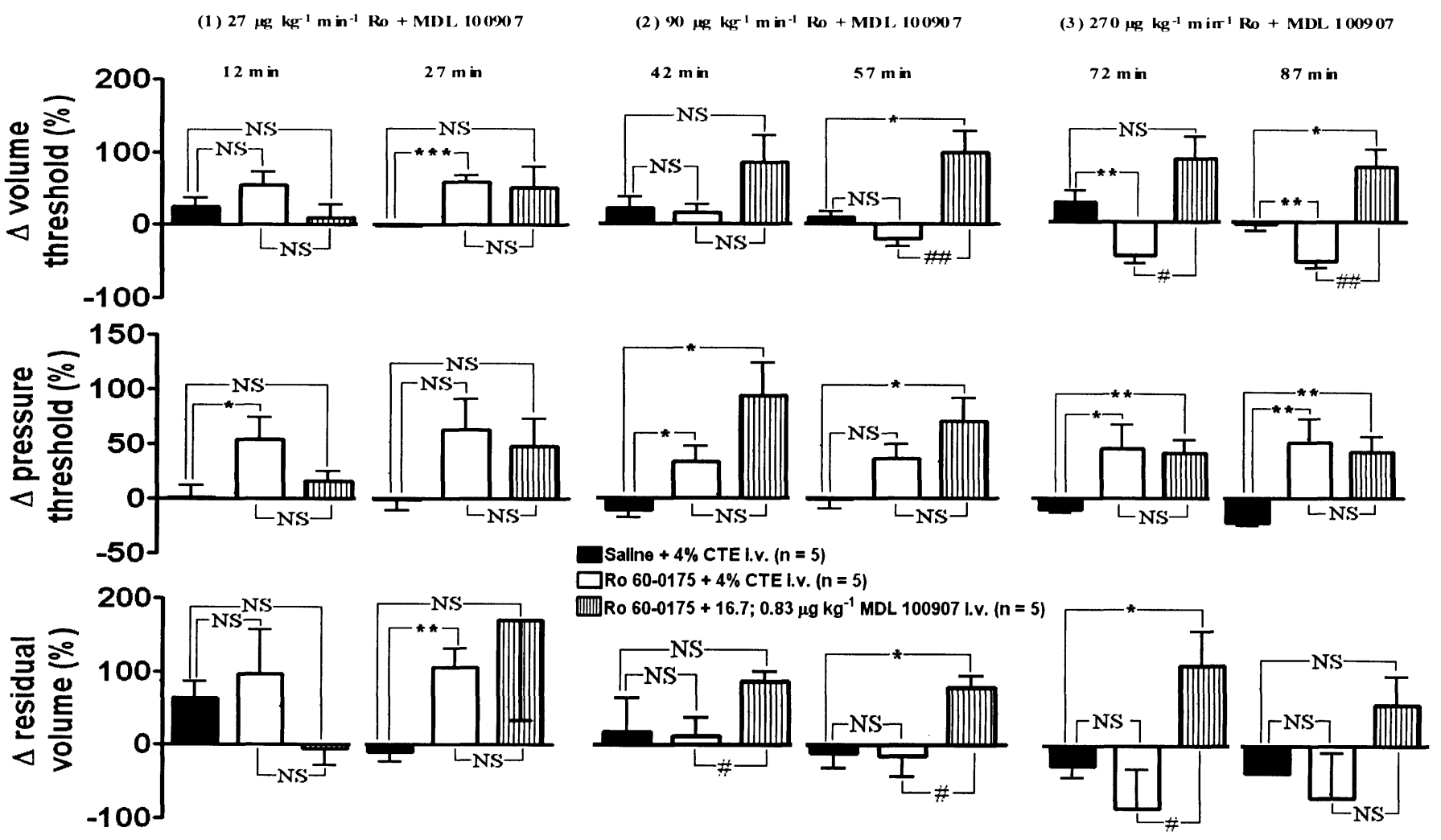

Figure 4.4c Urethane anaesthetised female rats: a comparison of the effects of infusion of vehicle (saline + 4% CTE), Ro 60-0175 and MDL 100907 on percentage changes (Δ) in volume threshold, pressure threshold and residual volume. Each bar represents the mean value and vertical bars show the s.e.mean. For the micturition reflex the first reflex was tested ~ 15 minutes after onset of infusion of each dose of Ro 60-0175 and the second reflex was tested ~30 minutes later (6 reflexes were tested in total i.e. 2 reflexes for each dose of Ro 60-0175 infused). Changes caused by Ro 60-0175 and MDL 100907 were compared with saline and 4% CTE control using Student's unpaired t-test. *,# $P<0.05$, **,## $P<0.01$, *** $P<0.001$, NS non-significant.

4.2.4 SB 242084 (5-HT_{2C} receptor antagonist)

Infusion of SB 242084 (* 22.5, 3.5 and 67.5, 3.5 μg kg^{-1} min^{-1}, i.v; n = 5) evoked no significant changes in baseline EUS-EMG signal, urethral pressure or the micturition reflex (Figure 4.5a & 4.5b).

The low infusion dose of SB 242084 (22.5; 3.5 μg kg^{-1} min^{-1}) caused a transient decrease in MAP (-4 ± 1 mmHg) which was found to be significant at the first minute but returned back to baseline soon after (Figure 4.5a). Even though the highest infusion dose of SB 242084 (67.5 μg kg^{-1} min^{-1}) caused a decrease in MAP (Figure 4.5a), this was found to be non-significant. Neither of the two doses of SB 242084 had any effect on HR.

* Doses of SB 242084 (both alone or as a combination with Ro 60-0175) were administered as loading doses of 22.5 and 67.5 μg kg^{-1} min^{-1} for 15 min and a maintenance dose of 3.5 μg kg^{-1} min^{-1} thereafter.

4.2.5 MDL 100907 (5-HT_{2A} receptor antagonist)

Infusion of MDL 100907 alone (*1.67, 0.83; 5.01, 0.83 and 16.7, 0.83 $\mu g\ kg^{-1}\ min^{-1}$; i.v; n = 5) evoked no significant changes in baseline EUS-EMG signal and urethral pressure (Figure 4.6a).

On the micturition reflex, only the lowest infusion dose of MDL 100907 (1.67, 0.83 $\mu g\ kg^{-1}\ min^{-1}$) caused a significant increase in volume threshold (30 ± 11%; reflex 2; Figure 4.6b), whereas the other two infusion doses had no significant effects on pressure threshold and residual volume.

All three doses of MDL 100907 caused significant decreases in MAP of -11 ± 0.2, -16 ± 2 and -25 ± 0.4 mmHg respectively with increasing dose (Figure 4.6a) but were ineffective on HR.

* Doses of MDL 100907 (both alone and as a combination with Ro 60-0175) were administered as loading doses of 1.67, 5.01 and 16.7 $\mu g\ kg^{-1}\ min^{-1}$ for 15 minutes and a maintenance dose of 0.83 $\mu g\ kg^{-1}\ min^{-1}$ thereafter.

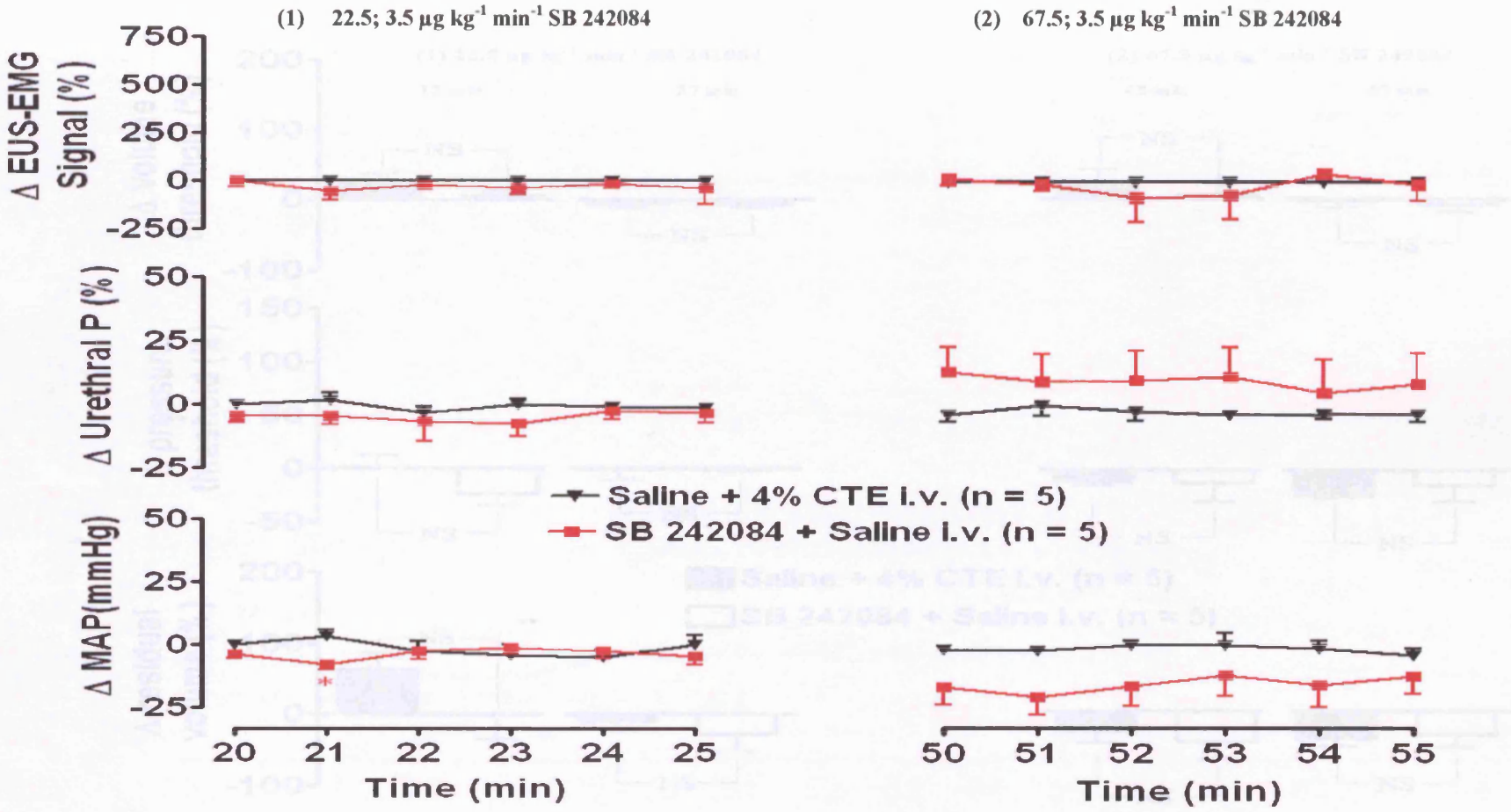

Figure 4.5a **Urethane anaesthetised female rats:** a comparison of the effects of infusion of SB 242084 and vehicle (saline + 4% CTE) on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (UP) and mean arterial blood pressure (MAP). Baseline variables were recorded for a duration of 5 min whilst the bladder was filled to 80% volume of saline required to elicit a micturition reflex. Panel (1) shows baseline variables recorded at 20-25 min after start of infusion of both SB 242084 (22.5; 3.5 µg kg^{-1} min^{-1}) and vehicle and Panel (2) shows variables at 50-55 min after start of infusion of both SB 242084 and vehicle. Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by SB 242084 were compared with vehicle using two-way analysis of variance followed by the least significant difference test. * $P<0.05$.

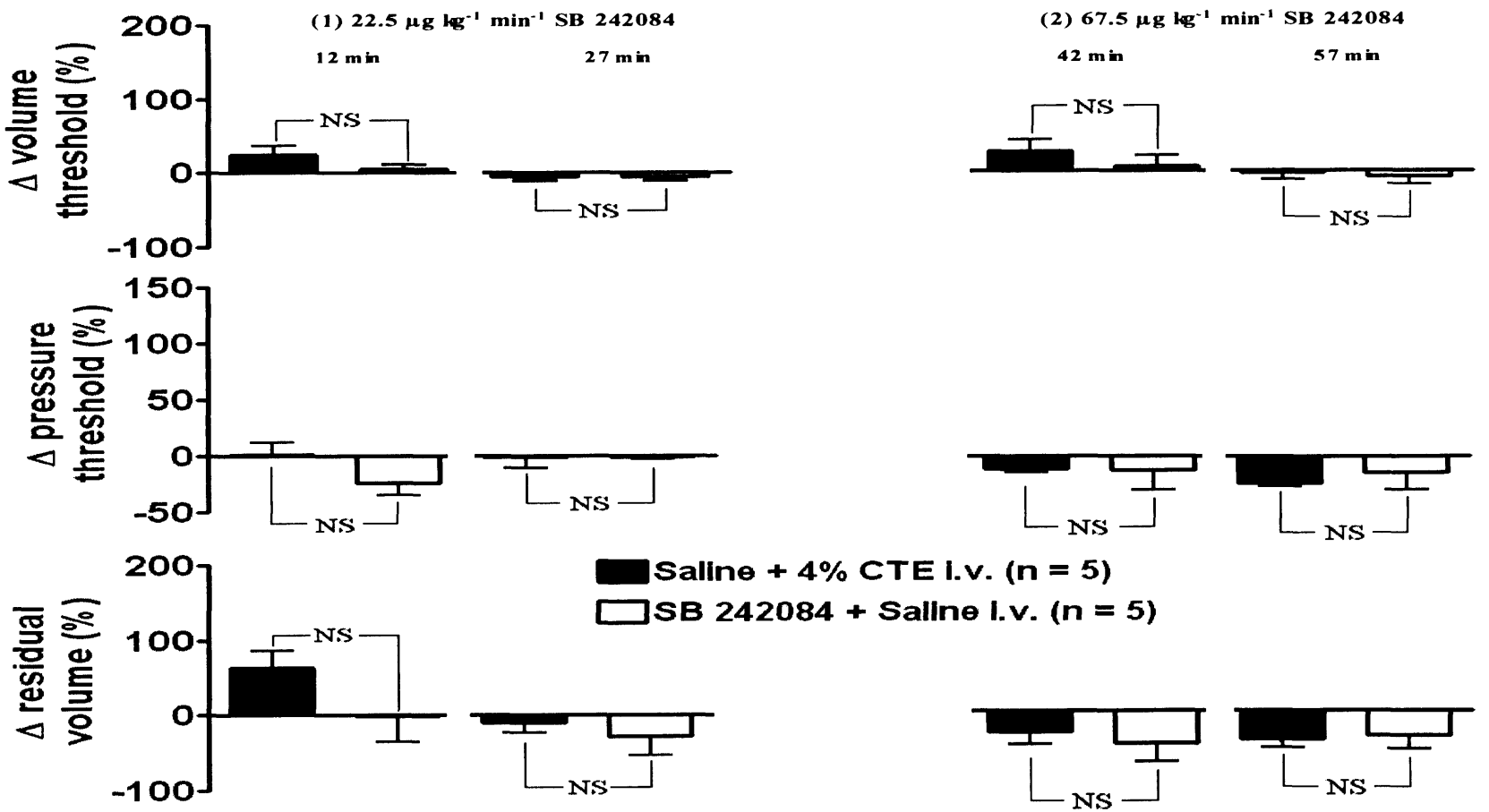

Figure 4.5b **Urethane anaesthetised female rats:** a comparison of the effects of infusion of SB 242084 and vehicle (saline + 4% CTE) on percentage changes (Δ) in volume threshold, pressure threshold and residual volume. Two micturition reflexes were tested for each infusion dose (~ 30 min duration for each infusion dose). Panel (1) shows variables recorded from testing the micturition reflex at 12 and 27 min after start of infusion of both SB 242084 (22.5; 3.5 µg kg^{-1} min^{-1}) and vehicle and Panel (2) shows variables from testing the micturition reflex at 42 and 57 min after start of infusion of SB 242084 and vehicle. Each bar represents the mean value and vertical bars show the s.e.mean. Changes caused by SB 242084 were compared with vehicle using Student's unpaired t-test. NS non-significant.

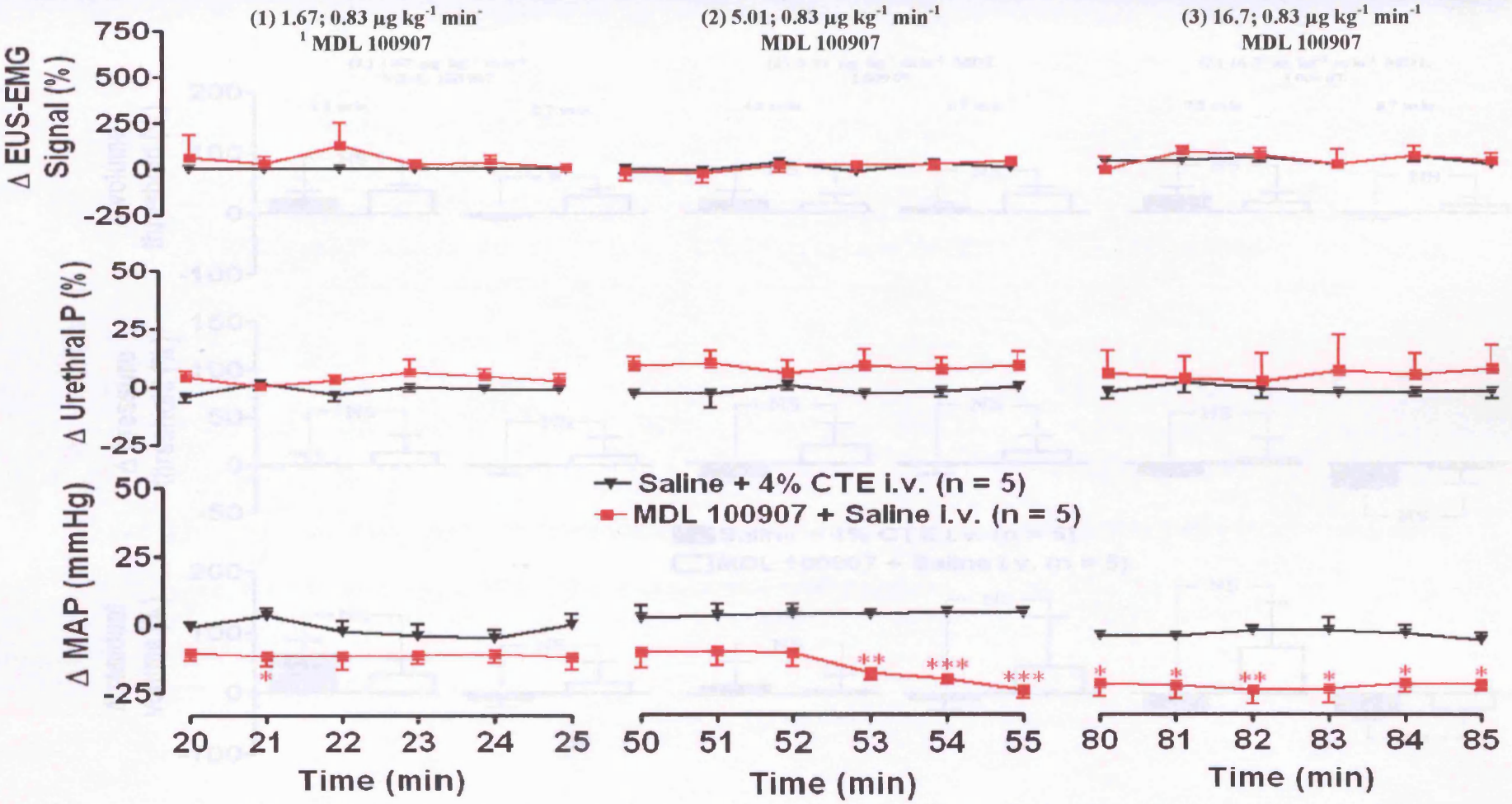

Figure 4.6a **Urethane anaesthetised female rats:** a comparison of the effects of infusion of MDL 100907 and vehicle (saline + 4% CTE) on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (P) and mean arterial blood pressure (MAP). Baseline variables were recorded for a duration of 5 min whilst the bladder was filled to 80% volume of saline required to elicit a micturition reflex. Panel (1) shows baseline variables recorded at 20-25 min after start of infusion of both MDL 100907 (1.67; 0.83 µg kg^{-1} min^{-1}) and vehicle, Panel (2) shows variables at 50-55 min after start of infusion of first dose of MDL 100907 and vehicle and Panel (3) shows variables at 80-85 min after start of infusion of first dose of MDL 100907 and vehicle. Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by MDL 100907 were compared with vehicle control using two-way analysis of variance followed by the least significant difference test. * P<0.05, ** P<0.01, *** P<0.001.

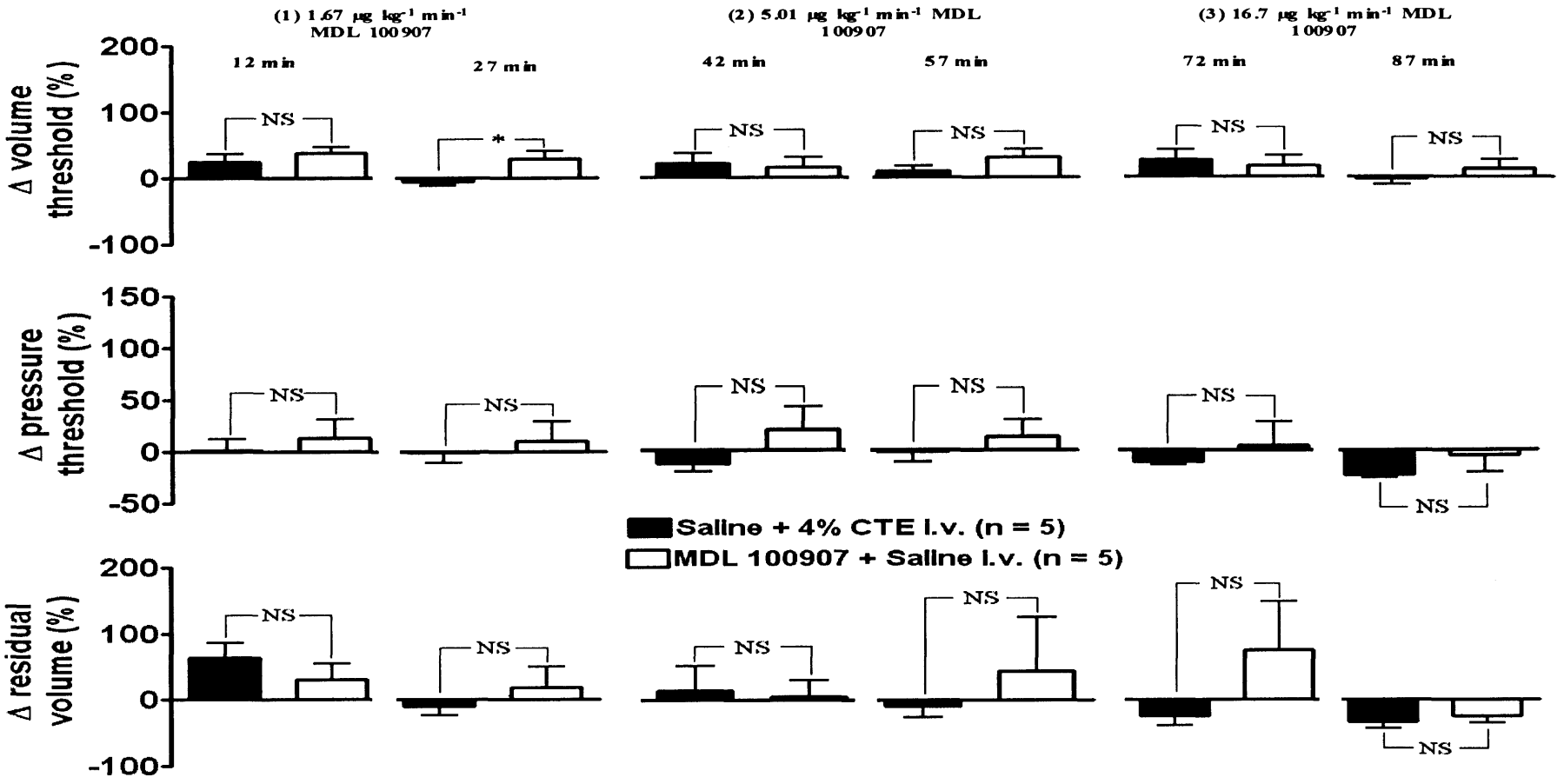

Figure 4.6b **Urethane anaesthetised female rats:** a comparison of the effects of infusion of MDL 100907 and vehicle (saline + 4% CTE) on percentage changes (Δ) in volume threshold, pressure threshold and residual volume. Two micturition reflexes were tested for each infusion dose (~ 30 min duration for each infusion dose). Panel (1) shows variables recorded from testing the micturition reflex at 12 and 27 min after start of infusion of both MDL 100907 (1.67; 0.83 µg kg^{-1} min^{-1}) and vehicle, Panel (2) shows variables from testing the micturition reflex at 42 and 57 min after start of infusion of MDL 100907 and vehicle and Panel (3) shows variables from testing the micturition reflex at 72 and 87 min after start of infusion of MDL 100907 and vehicle. Each bar represents the mean value and vertical bars show the s.e.mean. Changes caused by MDL 100907 were compared with vehicle using Student's unpaired t-test. *P<0.05, NS non-significant.

Ro 60-0175 27, 90, 270 μg kg^{-1} min^{-1} (n = 5)				SB 242084 22.5; 3.5 μg kg^{-1} min^{-1} (n = 5)			MDL 100907 16.7; 0.83 μg kg^{-1} min^{-1} (n = 3)		
Target [Free Plasma] nM	**10**	**30**	**100**	**3**			**3**		
Measured [Free Plasma] nM	8	31	110	5	5	4	15	5	3

Table 4.1 Summary of the measured free plasma concentrations of 5-HT_{2C} receptor agonist Ro 60-0175, 5-HT_{2C} receptor antagonist SB 242084 and 5-HT_{2A} receptor antagonist MDL 100907 administered as infusion doses i.v.

Drug & treatment	Receptor selectivity		EUS EMG	Urethral pressure	Micturition Reflex				Cardiovascular effects	
					Overall	Vol T	Pressure T	Residual Vol	Blood Pressure	Heart Rate
Ro 60-0175 (sal)										
27 μg kg^{-1} min^{-1}	5-HT_{2C}	agonist	↔	↔	inhibitory	↑↑	↑	↔	↔	↔
90 μg kg^{-1} min^{-1}			↔	↔	excitatory (?)	↓	↔	↔	↔	↔
270 μg kg^{-1} min^{-1}			↑	↔	excitatory	↓↓	↔	↔	↔	↔
Ro 60-0175 (4% CTE)										
27 μg kg^{-1} min^{-1}			↔	↔	inhibitory	↑↑↑	↑	↑↑	↔	↔
90 μg kg^{-1} min^{-1}			↔	↔	excitatory (?)	↔	↑	↔	↔	↔
270 μg kg^{-1} min^{-1}			↑↑↑	↔	excitatory	↓↓	↑↑	↔	↑↑↑	↔
SB 242084										
22.5; 3.5 μg kg^{-1} min^{-1}	5-HT_{2C}	antagonist	↔	↔	no effect	↔	↔	↔	↔	↔
67.5; 3.5 μg kg^{-1} min^{-1}			↔	↔		↔	↔	↔	↔	↔
MDL 100907										
1.67; 0.83 μg kg^{-1} min^{-1}	5-HT_{2A}	antagonist	↔	↔	Inhibitory (?)	↑	↔	↔	↓	↔
5.01; 0.83 μg kg^{-1} min^{-1}			↔	↔	no effect	↔	↔	↔	↓↓↓	↔
16.7; 0.83 μg kg^{-1} min^{-1}			↔	↔	no effect	↔	↔	↔	↓↓	↔

Table 4.2a Summary of 5-HT_2 receptor agonist and/or antagonist evoked responses (following i.v. infusions) on EUS-EMG, urethral pressure, micturition reflex and cardiovascular effects

Drug & treatment	Receptor selectivity		EUS EMG	Urethral pressure	Micturition Reflex				Cardiovascular effects	
					Overall	Vol T	Pressure T	Residual Vol	Blood Pressure	Heart Rate
Ro (27) + SB (22.5; 3.5)			↔	↔	excitatory	↓	↔	↓↓	↔	↔
Ro (90) + SB (22.5; 3.5)			↔	↔	excitatory	↓↓	↑	↓	↔	↔
Ro (270) + SB (22.5; 3.5)			↑↑↑	↔	excitatory	↓↓	↑↑↑	↔	↑↑	↔
Ro (27) + SB (67.5; 3.5)										
Ro (90) + SB (67.5; 3.5)			↔	↔	excitatory	↓	↓	↓↓	↔	↔
Ro (270) + SB (67.5; 3.5)			↔	↔	excitatory	↓↓	↔	↓	↔	↔
			↑↑↑	↔	excitatory	↓↓	↑	↔	↑↑	↔
Ro (27) + MDL (16.7; 0.83)			↔	↔	no effect	↔	↔	↔	↓↓↓	↔
Ro (90) + MDL (16.7; 0.83)			↔	↔	inhibitory	↑	↑	↑	↓	↔
Ro (270) + MDL (16.7; 0.83)			blocked	↔	inhibitory	↑	↑↑	↑	↓↓↓	↔

Table 4.2b Summary of the effects of i.v. infusion of Ro 60-0175 + SB 242084 and Ro 60-0175 + MDL 100907 on EUS-EMG activity, urethral pressure, micturition reflex and cardiovascular effects.

Experimental Group	n	Onset time of EUS-EMG activity (s)
Ro 60-0175 + Saline (270 μg kg^{-1} min^{-1})	5	92 ± 25
Ro 60-0175 + 4% CTE (270 μg kg^{-1} min^{-1})	5	83 ± 14
Ro; 270 μg kg^{-1} min^{-1} + SB 242084 (22.5; 3.5 μg kg^{-1} min^{-1})	5	100 ± 27
Ro; 270 μg kg^{-1} min^{-1} + SB 242084 (67.5; 3.5 μg kg^{-1} min^{-1})	3	113 ± 16

Table 4.3 Summary of onset times of appearance of EUS-EMG activity following Ro 60-0175 + saline, Ro 60-0175 + 4% CTE, Ro 60-0175 + SB 242084 (22.5; 3.5 and 67.5; 3.5 μg kg^{-1} min^{-1}). All values are expressed as mean ± sem. No statistical differences were observed when comparisons were made between the different groups.

4.3 Discussion

Infusion of both the low and middle dose of Ro 60-0175 did not evoke any EUS-EMG activity, whereas the highest dose evoked EUS-EMG activity. Additionally, none of the three doses of Ro 60-0175 had any effect on urethral pressure in contrast to the increase seen after bolus doses. On the micturition reflex, the low dose of Ro 60-0175 was found to be inhibitory, however as the dose was gradually increased, volume threshold decreased but pressure threshold remained elevated. It should be noted that on the micturition reflexes, differences were observed on pressure threshold following infusion of Ro 60-0175 and saline and Ro 60-0175 and 4% CTE. Infusion of Ro 60-0175 with 4% CTE was observed to cause significant increases in pressure threshold, whereas infusion of Ro 60-0175 with saline was ineffective on pressure threshold. This is interesting as it suggests the possibility that CTE may have caused a greater access of Ro 60-0175 to the receptor sites that when activated resulted in an increase in pressure threshold. Reflex evoked EUS-EMG activity and urethral relaxations were observed to be unaffected following infusions of Ro 60-0175, as reported for bolus doses in chapter 3. Ro 60-0175 has been characterized with full agonist efficacy on the 5-HT_{2C} receptor (Ki = 1 nM) as compared to the 5-HT_{2A} receptor (Ki = 32 nM; Hemrick-Luecke & Evans, 2002) in cloned rat binding studies and is therefore described as a 5-HT_{2C} receptor preferring agonist (Millan *et al.*, 1997). Thus it would be expected that the low dose effects are mediated by 5-HT_{2C} receptors and the high dose effects are mediated by 5-HT_{2A} receptors. This data would therefore be consistent with the view that activating 5-HT_{2C} receptors has an inhibitory action on the micturition reflex whereas EUS-EMG activation is mediated via the 5-HT_{2A} receptor (as this effect was only seen with the highest infusion rate of Ro 60-0175). To further confirm these conclusions, infusion of these three doses of

Ro 60-0175 was studied in the presence of 5-HT_{2C} or 5-HT_{2A} receptor antagonists SB 242084 or MDL 100907 respectively. The antagonists were given as initial loading dose followed by maintenance infusions (see Methods, Chapter 2). Both doses of SB 242084 failed to block the highest infusion dose of Ro 60-0175 evoked EUS-EMG activity. Interestingly, the inhibitory action of the low dose of Ro 60-0175 on the micturition reflex was blocked; with a tendency to now observe an excitatory action, which at least for volume threshold became more prominent as the dose increased. This would be consistent with the view that 5-HT_{2A} receptors are excitatory on the micturition reflex (Chapter 3) and with the MDL 100907 infusion data showing a significant increase in volume threshold, at least with the lower dose of MDL 100907. Moreover, the effects of Ro 60-0175 on volume threshold were not paralleled by similar effects on pressure threshold, which was only significantly blocked by the higher dose of SB 242084. It is possible for volume threshold to change independently of pressure threshold as volume threshold changes reflect changes in compliance of the bladder i.e. smooth muscle resting tone. Thus an increase in pressure threshold would more than likely indicate central inhibition of the reflexes, a decrease in bladder compliance on an increase in EUS tone, whereas an increase in volume threshold would reflect changes in one or more of the following, sympathetic drive, parasympathetic drive and/or a direct effect on smooth muscle tone or a peripherally or centrally-mediated reduction in bladder afferent sensitivity. Thus peripheral activation of 5-HT_{2A} receptors on bladder smooth muscle would be expected to decrease volume threshold. However, the present data does not support an action of Ro 60-0175 activating central 5-HT_{2A} receptors, which are believed to be excitatory on the micturition reflex, but peripheral 5-HT_{2A} receptors on bladder smooth muscle. It could be considered that the initial reversal of volume threshold

occurs following blockade of the 5-HT_{2C} receptor inhibitory action, which would potentiate the underlying 5-HT_{2A} receptor mediated increase in bladder tone. This would explain why it occurs at lower doses of SB 242084 compared with inhibition of the increase in pressure threshold which is affected by 5-HT_{2C} receptor activation. Further, data from the blood pressure changes indicate that SB 242084 at the doses chosen failed to affect the evoked rise in blood pressure by Ro 60-0175, which is mediated by peripheral 5-HT_{2A} receptors causing constriction of vascular smooth muscle (see Chapter 5). This would also indicate that the doses chosen for SB 242084 do not interfere with 5-HT_{2A} receptors as indicated by Pfizer in house calculations. Moreover, the failure of SB 242084 at the high dose infusion alone to affect any other variables being measured indicates that 5-HT_{2C} receptors are not physiologically involved in micturition in the anaesthetised rat as proposed by de Groat (2002), (see introduction).

Conversely, the selective 5-HT_{2A} receptor antagonist MDL 100907 caused blockade of the highest infusion dose Ro 60-0175 evoked increase in EUS-EMG activity. In addition, Ro 60-0175 in the presence of MDL 100907 failed to evoke a decrease in volume threshold, with the evoked increase in pressure threshold unaffected. MDL 100907 alone had no effect on resting variables and little effect on the micturition reflex apart from a small increase in volume threshold at the lowest dose. Thus this data demonstrates that in the rat, activation of 5-HT_{2A} receptors causes EUS-EMG activity, and confirms the view that activation of central 5-HT_{2C} receptors have a small inhibitory role on the micturition reflex. However, there is little or no evidence from the present infusion study that either of these receptors play a physiological role in micturition. Interestingly, infusion of MDL 100907 did cause a slow time related

and thus dose related fall in blood pressure suggesting that these receptors may receive a central tonic excitatory drive. However, why the bolus dose of MDL 100907 increased blood pressure remains to be determined, although this may again relate to a high free plasma concentration being obtained, that blocks other receptor subtypes.

As urethral pressure was observed to be unaffected in the present data, it would appear that neither 5-HT_{2A} nor 5-HT_{2C} receptors are responsible in mediating this effect. Data from the previous chapter suggested the involvement of 5-HT_{2B} receptors in mediating the increases observed in urethral pressure, as the 5-HT_{2B} receptor antagonist RS 127445 reversed the Ro 60-0175 evoked increase in urethral pressure. Although there is no evidence in the literature examining a physiological role for 5-HT_{2B} receptors in the control of urethral smooth muscle function, these receptors have been implicated in gastric smooth muscle contraction (Kitazawa *et al.*, 2006). Indeed the 5-HT_{2B} receptor subtype is highly expressed in the rat stomach fundus and has been proposed as being functional in mediating gastric smooth muscle contractions. The actions of the 5-HT_{2B} receptor on gastric smooth muscle are thought to occur via Gq-coupled activation of phospholipase C and intracellular calcium mobilization (Kelly & Sharif, 2006). It is therefore possible that 5-HT_{2B} receptors cause urethral smooth muscle contraction via a mechanism similar to that mediating gastric smooth muscle contraction. However, in the present study, it would appear that slow infusions of Ro 60-0175 at the specified doses failed to activate 5-HT_{2B} receptors and the mechanism mediating urethral smooth muscle contraction.

Overall, the present data suggests that in the rat 5-HT_{2A} receptors are involved in activation of the external urethral sphincter and excitation of the micturition reflex whereas 5-HT_{2C} receptors are inhibitory on the micturition reflex. Additionally, neither 5-HT_{2A} nor 5-HT_{2C} receptors were found to be involved in mediating urethral pressure increases as previously observed in chapter 3 although the bolus data suggest that the actions on urethral pressure are mediated via 5-HT_{2B} receptors. Conclusions that 5-HT_{2A} receptors are involved in activation of the EUS is in agreement with previous studies carried out in the cat where it was found that 5-HT_{2A} receptor agonists facilitated EUS activity, where i.v. administration of the 5-HT_{2A} receptor agonist DOI was observed to increase pudendal reflexes (Thor & Katofiasc, 1995; Danuser *et al.*, 1995).

There has been speculation as to the specific role played by the external urethral sphincter in the rat i.e. is it mainly involved in micturition or continence. Indeed an increase in external urethral sphincter activity is representative of urethral sphincter contraction thus aiding in continence. Tracer studies have also revealed that injections of Pseudorabies virus into the external urethral sphincter infect the pontine storage center which directly projects to the Onuf's nucleus and innervates the external urethral sphincter via the pudendal nerve. However, whilst in the guinea pig and humans the external urethral sphincteric activity is completely inhibited immediately prior to urethral relaxation and bladder emptying, this does not occur in the rat, but rather high frequency bursts in EUS-EMG activity are observed during urethral relaxation (Walters *et al.*, 2006). These observations in the rat demonstrate a favourable role for the external urethral sphincter in enabling efficient voiding to

occur, whereas in the guinea pig and humans the external urethral sphincter is mainly involved in continence.

On the micturition reflex, the inhibitory effect of 5-HT_{2C} receptors and excitatory effects of 5-HT_{2A} receptors concur with that of the previous chapter. Additionally, these inhibitory effects are in agreement with previous published data by Steers & de Groat (1989) and Guarneri *et al* (1996) in rat studies. Moreover, a similar inhibitory action of 5-HT_{2C} receptor agonists on micturition in guinea pigs (McMurray & Miner, 2005) was also observed therefore suggesting that the mechanism for micturition in both species may be very similar with an influence on both parasympathetic and sympathetic outputs to the bladder.

4.3.3 Is there evidence of 'desensitisation' following continuous infusion of 5-$HT_{2A/2C}$ receptor ligands

Considerations as to the possibility of desensitisation should be taken into account in the present study due to the fact that the protocol utilized a continuous infusion dosing regimen. Evidence for desensitisation is mainly demonstrated by a decrease in compound efficacy. Although poorly understood, several mechanisms have been postulated as giving rise to desensitisation. These include a change or loss of the receptors, exhaustion of mediators, increased metabolic degradation and physiological adaptation (Rang *et al.*, 1995). Continuous intravenous infusion of 5-$HT_{2A/2C}$ receptor ligands in the present experiments failed to provide evidence for desensitisation of the 5-HT_2 receptors. Measures of free plasma concentrations of compounds remained elevated with no decline observed even towards the end of the experiment 90 min later from start of infusion. Moreover, compound efficacy was maintained with

regards to lower urinary tract function although infusion of the low and high dose of Ro 60-0175 presented opposing effects on the bladder and urethra. At the high dose, Ro 60-0175 appeared to still be functional due to the fact that evoked increases in EUS-EMG activity were observed (suggesting activation of a specific mechanism had taken place) which would not be the case had desensitisation occurred. Indeed to fully confirm a lack of desensitisation in the present study, further experiments infusing just the largest dose of Ro 60-0175 would help further characterise and confirm the actions of this dose on EUS-EMG activity and the micturition reflex.

4.4 Conclusion

From the present infusion study, it can be concluded that 5-HT_{2A} receptors are involved in mediating excitatory actions on the external urethral sphincter and the micturition reflex, whereas 5-HT_{2C} receptors are inhibitory on the micturition reflex. Additionally, neither of these two receptor subtypes are involved in reflex evoked urethral muscle regulation during micturition, nor are they involved in mediating the urethral smooth muscle contractions observed in chapter 3. Additional confirmation for the roles of both the 5-HT_{2A} and 5-HT_{2C} receptors in mediating their respective bladder and urethral functions is provided from information obtained following measures of free plasma concentration of the compounds, of which selectivity of the receptor subtype was achieved following infusion of the compound at a specific target concentration. Moreover, evidence for a facilitatory role of 5-HT_{2A} receptors in micturition rather than continence in the female rat is also demonstrated in this study.

Experimental group	n	EUS-EMG			Urethral Pressure (UP)			Mean Arterial Pressure (MAP)			Heart Rate (HR)		
		Baseline μV	Test μV	Δ %	Baseline mmHg	Test mmHg	Δ %	Baseline mmHg	Test mmHg	Δ mmHg	Baseline bpm	Test bpm	Δ bpm
Saline (First dose infusion)	5	0.15 ± 0.08	0.20 ± 0.60	-	18 ± 0.1	18 ± 0.3	-5 ± 2	97 ± 0.2	101 ± 1	5 ± 0.5	356 ± 5	359 ± 2	3 ± 3
Saline (Second dose infusion)	5	0.15 ± 0.08	0.10 ± 0.02	-	18 ± 0.1	17 ± 0.1	-17 ± 1	97 ± 0.2	90 ± 0.5	-7 ± 0.4	356 ± 5	360 ± 4	4 ± 2
Saline (Third dose infusion)	5	0.15 ± 0.08	0.13 ± 0.02	-	18 ± 0.1	18 ± 0.2	-7 ± 3	97 ± 0.2	84 ± 1	-13 ± 1	356 ± 5	355 ± 4	2 ± 1
Saline + 4% CTE (First dose infusion)	5	0.27 ± 0.02	0.39 ± 0.05	-	27 ± 0.1	27 ± 0.2	-1 ± 1	97 ± 0.2	96 ± 1	-2 ± 1.0	348 ± 3	350 ± 2	2 ± 3
Saline + 4% CTE (Second dose infusion)	5	0.27 ± 0.02	0.29 ± 0.01	-	27 ± 0.1	27 ± 0.1	-1 ± 1	97 ± 0.2	98 ± 0.3	1 ± 0.3	348 ± 3	355 ± 3	7 ± 3
Saline + 4% CTE (Third dose infusion)	5	0.27 ± 0.02	0.24 ± 0.10	-	27 ± 0.1	27 ± 0.1	-2 ± 0.5	97 ± 0.2	86 ± 0.5	-4 ± 0.4	348 ± 3	357 ± 2	9 ± 4

Table 4.4a Vehicle (Saline alone or Saline combined with 4% CTE) i.v. infusion experiments in urethane anaesthetized rats: baseline and test values and changes (Δ) in external sphincter (EUS) EMG, urethral pressure (UP), mean blood pressure (MAP) and heart rate (HR)

Experimental Group	n	Volume Threshold				Pressure Threshold				Residual Volume			
		Baseline ml		Test ml	Δ %	Baseline mmHg		Test mmHg	Δ %	Baseline ml		Test ml	Δ %
Saline (First dose infusion)	5	0.40 ± 0.03	r1	0.46 ± 0.04	17 ± 6	8 ± 1	r1	7 ± 1	-5 ± 4	0.18 ± 0.03	r1	0.18 ± 0.05	-9 ± 13
			r2	0.50 ± 0.06	38 ± 18		r2	9 ± 1	20 ± 14		r2	0.29 ± 0.06	85 ± 52
Saline (Second dose infusion)	5	0.40 ± 0.03	r1	0.50 ± 0.04	28 ± 3	8 ± 1	r1	8 ± 1	-1 ± 8	0.18 ± 0.03	r1	0.21 ± 0.05	12 ± 27
			r2	0.45 ± 0.06	15 ± 6		r2	8 ± 1	4 ± 8		r2	0.20 ± 0.05	-20 ± 49
Saline (Third dose infusion)	5	0.40 ± 0.03	r1	0.46 ± 0.05	16 ± 7	8 ± 1	r1	7 ± 1	-16 ± 6	0.18 ± 0.03	r1	0.11 ± 0.03	-36 ± 11
			r2	0.50 ± 0.06	28 ± 12		r2	8 ± 1	-3 ± 10		r2	0.12 ± 0.04	-37 ± 12
Saline + 4% CTE (First dose infusion)	5	0.49 ± 0.11	r1	0.60 ± 0.12	25 ± 12	11 ± 1	r1	11 ± 1	1 ± 11	0.25 ± 0.09	r1	0.35 ± 0.08	64 ± 23
			r2	0.47 ± 0.10	-5 ± 7		r2	10 ± 1	-1 ± 10		r2	0.22 ± 0.07	-10 ± 13
Saline + 4% CTE (Second dose infusion)	5	0.49 ± 0.11	r1	0.62 ± 0.18	22 ± 14	11 ± 1	r1	10 ± 1	-11 ± 8	0.25 ± 0.09	r1	0.3 ± 0.14	15 ± 35
			r2	0.52 ± 0.10	10 ± 8		r2	11 ± 2	-1 ± 9		r2	0.23 ± 0.1	-6 ± 17
Saline + 4% CTE (Third dose infusion)	5	0.49 ± 0.11	r1	0.63 ± 0.17	26 ± 16	11 ± 1	r1	10 ± 1	-11 ± 3	0.25 ± 0.09	r1	0.23 ± 0.14	-25 ± 14
			r2	0.46 ± 0.08	-2 ± 9		r2	8 ± 1	-22 ± 4		r2	0.17 ± 0.07	-33 ± 11

Table 4.4b Vehicle (Saline alone or Saline combined with 4% CTE) i.v. experiments in urethane anaesthetized rats: baseline and test values and changes (Δ) in volume threshold, pressure threshold and residual volume. r1 and r2 represents reflex 1 and reflex 2.

Experimental group	n	EUS-EMG			Urethral Pressure (UP)			Mean Arterial Pressure (MAP)			Heart Rate (HR)		
		Baseline μV	Test μV	Δ %	Baseline mmHg	Test mmHg	Δ %	Baseline mmHg	Test mmHg	Δ mmHg	Baseline bpm	Test bpm	Δ bpm
Ro 60-0175 + Saline (27 μg kg^{-1} min^{-1})	6	0.49 ± 0.07	0.64 ± 0.30	-	16 ± 0.1	18 ± 0.1	4 ± 1	97 ± 0.3	94 ± 0.6	-3 ± 0.1	389 ± 0.4	392 ± 2	3 ± 2
Ro 60-0175 + Saline (90 μg kg^{-1} min^{-1})	6	0.49 ± 0.07	0.80 ± 0.14	-	16 ± 0.1	18 ± 0.1	3 ± 1	97 ± 0.3	97 ± 0.4	0.4 ± 0.4	389 ± 0.4	410 ± 2	22 ± 2
Ro 60-0175 + Saline (270 μg kg^{-1} min^{-1})	5	0.49 ± 0.07	1.70 ± 0.12	144 ± 11	16 ± 0.1	15 ± 0.2	-28 ± 2	97 ± 0.3	100 ± 0.3	3 ± 1	389 ± 0.4	406 ± 1	13 ± 1
Ro 60-0175 + 4% CTE (27 μg kg^{-1} min^{-1})	5	0.22 ± 0.06	0.37 ± 0.06	-	23 ± 0.1	25 ± 0.2	2 ± 0.3	111 ± 2	113 ± 0.1	0.2 ± 0.4	345 ± 2	362 ± 1	17 ± 2
Ro 60-0175 + 4% CTE (90 μg kg^{-1} min^{-1})	5	0.22 ± 0.06	0.90 ± 0.65	45 ± 6	23 ± 0.1	25 ± 0.09	5 ± 0.7	111 ± 2	122 ± 0.2	9 ± 0.5	345 ± 2	387 ± 1	42 ± 2
Ro 60-0175 + 4% CTE (270 μg kg^{-1} min^{-1})	5	0.22 ± 0.06	1.10 ± 0.10	1093 ± 141	23 ± 0.1	23 ± 0.1	-3 ± 1	111 ± 2	132 ± 0.3	19 ± 1	345 ± 2	364 ± 4	19 ± 3

Table 4.5a Ro 60-0175 + Saline and Ro 60-0175 + 4% CTE i.v. infusion experiments in urethane anaesthetized rats: baseline and test values and changes (Δ) in external sphincter (EUS) EMG, urethral pressure (UP), mean blood pressure (MAP) and heart rate (HR)

Experimental Group	n	Volume Threshold				Pressure Threshold				Residual Volume			
		Baseline ml	Test ml		Δ %	Baseline mmHg	Test mmHg		Δ %	Baseline ml	Test ml		Δ %
Ro 60-0175 + Saline (27 μg kg^{-1} min^{-1})	6	0.38 ± 0.04	r1	0.61 ± 0.09	68 ± 10	9 ± 1	r1	11 ± 1	21 ± 8	0.19 ± 0.03	r1	0.26 ± 0.06	90 ± 80
			r2	0.65± 0.04	74 ± 14		r2	12 ± 2	37 ± 25		r2	0.32 ± 0.06	81 ± 29
Ro 60-0175 + Saline (90 μg kg^{-1} min^{-1})	6	0.38 ± 0.04	r1	0.42 ± 0.04	15 ± 19	9 ± 1	r1	12 ± 1	35 ± 23	0.19 ± 0.03	r1	0.17 ± 0.04	-5 ± 17
			r2	0.27 ± 0.03	-24 ± 14		r2	12 ± 1	31 ± 16		r2	0.14 ± 0.03	-24 ± 13
Ro 60-0175 + Saline (270 μg kg^{-1} min^{-1})	5	0.38 ± 0.04	r1	0.18 ± 0.02	-51 ± 11	9 ± 1	r1	10 ± 2	13 ± 20	0.19 ± 0.03	r1	0.11 ± 0.02	-43 ± 8
			r2	0.20 ± 0.04	-45 ± 12		r2	8 ± 1	-9 ± 16		r2	0.12 ± 0.03	-40 ± 8
Ro 60-0175 + 4% CTE (27 μg kg^{-1} min^{-1})	5	0.47 ± 0.09	r1	0.69 ± 0.12	54 ± 18	9 ± 0.5	r1	14 ± 2	55 ± 19	0.23 ± 0.07	r1	0.39 ± 0.14	97 ± 59
			r2	0.75 ± 0.16	59 ± 8		r2	15 ± 3	63 ± 28		r2	0.45 ± 0.14	105 ± 27
Ro 60-0175 + 4% CTE (90 μg kg^{-1} min^{-1})	5	0.47 ± 0.09	r1	0.56 ± 0.14	16 ± 10	9 ± 0.5	r1	12 ± 1	33 ± 15	0.23 ± 0.07	r1	0.27 ± 0.12	10 ± 20
			r2	0.39 ± 0.10	-18 ± 11		r2	12 ± 2	36 ± 13		r2	0.19 ± 0.07	-11 ± 23
Ro 60-0175 + 4% CTE (270 μg kg^{-1} min^{-1})	5	0.47 ± 0.09	r1	0.24 ± 0.04	-43 ± 9	9 ± 0.5	r1	14 ± 2	46 ± 22	0.23 ± 0.07	r1	0.12 ± 0.03	-79 ± 50
			r2	0.21 ± 0.03	-50 ± 10		r2	14 ± 2	53 ± 22		r2	0.14 ± 0.03	-65 ± 57

Table 4.5b Ro 60-0175 + Saline and Ro 60-0175 + 4% CTE i.v. experiments in urethane anaesthetized rats: baseline and test values and changes (Δ) in volume threshold, pressure threshold and residual volume. r1 and r2 represents reflex 1 and reflex 2.

Experimental group	n	EUS-EMG			Urethral Pressure (UP)			Mean Arterial Pressure (MAP)			Heart Rate (HR)		
		Baseline μV	Test μV	Δ %	Baseline mmHg	Test mmHg	Δ %	Baseline mmHg	Test mmHg	Δ mmHg	Baseline bpm	Test bpm	Δ bpm
Ro; 27 μg kg^{-1} min^{-1} + SB 242084 (22.5; 3.5 μg kg^{-1} min^{-1})	5	0.40 ± 0.03	0.46 ± 0.02	-	26 ± 0.3	28 ± 0.1	7 ± 1	112 ± 0.2	114 ± 0.4	2 ± 0.4	369 ± 3	372 ± 3	3 ± 3
Ro; 90 μg kg^{-1} min^{-1} + SB 242084 (22.5; 3.5 μg kg^{-1} min^{-1})	5	0.40 ± 0.03	0.34 ± 0.42	-	26 ± 0.3	28 ± 0.1	7 ± 1	112 ± 0.2	119 ± 0.5	7 ± 0.4	369 ± 3	351 ± 2	-18 ± 3
Ro; 270 μg kg^{-1} min^{-1} + SB 242084 (22.5; 3.5 μg kg^{-1} min^{-1})	5	0.40 ± 0.03	0.87 ± 0.25	1381 ± 129	26 ± 0.3	27 ± 0.1	3 ± 1	112 ± 0.2	132 ± 0.4	20 ± 0.3	369 ± 3	373 ± 2	4 ± 2
Ro; 27 μg kg^{-1} min^{-1} + SB 242084 (67.5; 3.5 μg kg^{-1} min^{-1})	3	0.10 ± 0.01	0.10 ± 0.05	-	28 ± 0.2	29 ± 0.2	4 ± 1	99 ± 1	107 ± 0.3	9 ± 1	387 ± 1	365 ± 1	-22 ± 1
Ro; 90 μg kg^{-1} min^{-1} + SB 242084 (67.5; 3.5 μg kg^{-1} min^{-1})	3	0.10 ± 0.01	0.10 ± 0.05	-	28 ± 0.2	29 ± 0.2	2 ± 1	99 ± 1	111 ± 1	12 ± 1	387 ± 1	342 ± 2	-44 ± 2
Ro; 270 μg kg^{-1} min^{-1} + SB 242084 (67.5; 3.5 μg kg^{-1} min^{-1})	3	0.10 ± 0.01	1.50 ± 0.05	1259 ± 88	28 ± 0.2	28 ± 0.1	-1 ± 1	99 ± 1	118 ± 1	20 ± 1	387 ± 1	340 ± 1	-46 ± 2

Table 4.6a Ro 60-0175 + SB 242084 (22.5 and 67.5 μg kg^{-1} min^{-1}) i.v. infusion experiments in urethane anaesthetized rats: baseline and test values and changes (Δ) in external sphincter (EUS) EMG, urethral pressure (UP), mean blood pressure (MAP) and heart rate (HR)

Experimental Group	n	Volume Threshold				Pressure Threshold				Residual Volume			
		Baseline ml		Test ml	Δ %	Baseline mmHg		Test mmHg	Δ %	Baseline ml		Test ml	Δ %
Ro; 27 μg kg^{-1} min^{-1} + SB 242084 (22.5; 3.5 μg kg^{-1} min^{-1})	5	0.43 ± 0.11	r1	0.45 ± 0.07	54 ± 45	9 ± 1	r1	12 ± 1	37 ± 23	0.26 ± 0.09	r1	0.20 ± 0.04	18 ± 54
			r2	0.31 ± 0.08	-29 ± 6		r2	10 ± 1	14 ± 12		r2	0.17 ± 0.05	-29 ± 12
Ro; 90 μg kg^{-1} min^{-1} + SB 242084 (22.5; 3.5 μg kg^{-1} min^{-1})	5	0.43 ± 0.11	r1	0.25 ± 0.06	-40 ± 6	9 ± 1	r1	10 ± 1	21 ± 11	0.26 ± 0.09	r1	0.11 ± 0.03	-46 ± 14
			r2	0.23 ± 0.05	-44 ± 6		r2	11 ± 1	24 ± 4		r2	0.10 ± 0.02	-48 ± 13
Ro; 270 μg kg^{-1} min^{-1} + SB 242084 (22.5; 3.5 μg kg^{-1} min^{-1})	5	0.43 ± 0.11	r1	0.21 ± 0.04	-46 ± 10	9 ± 1	r1	14 ± 1	57 ± 13	0.26 ± 0.09	r1	0.10 ± 0.02	-49 ± 15
			r2	0.20 ± 0.04	-47 ± 10		r2	14 ± 1	65 ± 15		r2	0.14 ± 0.04	-21 ± 27
Ro; 27 μg kg^{-1} min^{-1} + SB 242084 (67.5; 3.5 μg kg^{-1} min^{-1})	3	0.31 ± 0.06	r1	0.25 ± 0.03	-16 ± 5	9 ± 1	r1	7 ± 1	-19 ± 6	0.11 ± 0.06	r1	0.05 ± 0.02	-36 ± 19
			r2	0.28 ± 0.05	-9 ± 3		r2	7 ± 1	-18 ± 11		r2	0.04 ± 0.01	-45 ± 23
Ro; 90 μg kg^{-1} min^{-1} + SB 242084 (67.5; 3.5 μg kg^{-1} min^{-1})	3	0.31 ± 0.06	r1	0.18 ± 0.06	-42 ± 7	9 ± 1	r1	8 ± 2	16 ± 13	0.11 ± 0.06	r1	0.04 ± 0.02	-62 ± 6
			r2	0.18 ± 0.04	-40 ± 5		r2	7 ± 2	-19 ± 15		r2	0.05 ± 0.02	-46 ± 19
Ro; 270 μg kg^{-1} min^{-1} + SB 242084 (67.5; 3.5 μg kg^{-1} min^{-1})	3	0.31 ± 0.06	r1	0.14 ± 0.04	-55 ± 5	9 ± 1	r1	9 ± 3	-4 ± 29	0.11 ± 0.06	r1	0.03 ± 0.01	-61 ± 9
			r2	0.12 ± 0.01	-60 ± 3		r2	9 ± 1	7 ± 11		r2	0.03 ± 0.01	-65 ± 8

Table 4.6b Ro 60-0175 + SB 242084 (22.5; 3.5 and 67.5; 3.5 μg kg^{-1} min^{-1}) i.v. experiments in urethane anaesthetized rats: baseline and test values and changes (Δ) in volume threshold, pressure threshold and residual volume. r1 and r2 represents reflex 1 and reflex 2.

Experimental group	n	EUS-EMG			Urethral Pressure (UP)			Mean Arterial Pressure (MAP)			Heart Rate (HR)		
		Baseline μV	Test μV	Δ %	Baseline mmHg	Test mmHg	Δ %	Baseline mmHg	Test mmHg	Δ mmHg	Baseline bpm	Test bpm	Δ bpm
Ro; 27 μg kg^{-1} min^{-1} + MDL 100907 (16.7; 0.83 μg kg^{-1} min^{-1})	5	0.30 ± 0.01	0.51 ± 0.04	-	25 ± 0.1	26 ± 0.1	1 ± 1	104 ± 0.3	88 ± 0.2	-16 ± 0.4	372 ± 1	362 ± 1	-8 ± 2
Ro; 90 μg kg^{-1} min^{-1} + MDL 100907 (16.7; 0.83 μg kg^{-1} min^{-1})	5	0.30 ± 0.01	0.35 ± 0.01	-	25 ± 0.1	27 ± 0.1	6 ± 1	104 ± 0.3	87 ± 0.4	-16 ± 1	372 ± 1	359 ± 1	-3 ± 1
Ro; 270 μg kg^{-1} min^{-1} + MDL 100907 (16.7; 0.83 μg kg^{-1} min^{-1})	5	0.30 ± 0.01	0.40 ± 0.02	-	25 ± 0.1	25 ± 0.1	-2 ± 0.4	104 ± 0.3	87 ± 0.2	-17 ± 0.3	372 ± 1	344 ± 3	-27 ± 3

Table 4.7a Ro 60-0175 + MDL 100907 (16.7; 0.83 μg kg^{-1} min^{-1}) i.v. infusion experiments in urethane anaesthetized rats: baseline and test values and changes (Δ) in external sphincter (EUS) EMG, urethral pressure (UP), mean blood pressure (MAP) and heart rate (HR)

Experimental Group	n	Volume Threshold				Pressure Threshold				Residual Volume			
		Baseline ml		Test ml	Δ %	Baseline mmHg		Test mmHg	Δ %	Baseline ml		Test ml	Δ %
Ro; 27 μg kg^{-1} min^{-1} + MDL 100907 (16.7; 0.83 μg kg^{-1} min^{-1})	5	0.44 ± 0.06	r1	0.48 ± 0.10	10 ± 17	9 ± 1	r1	10 ± 1	16 ± 9	0.21± 0.07	r1	0.19 ± 0.08	-5 ± 23
			r2	0.63 ± 0.11	51 ± 29		r2	13 ± 2	48 ± 25		r2	0.40 ± 0.15	170 ± 136
Ro; 90 μg kg^{-1} min^{-1} + MDL 100907 (16.7; 0.83 μg kg^{-1} min^{-1})	5	0.44 ± 0.06	r1	0.83 ± 0.07	84 ± 35	9 ± 1	r1	17 ± 1	93 ± 30	0.21± 0.07	r1	0.63 ± 0.04	68 ± 10
			r2	0.80 ± 0.08	97 ± 28		r2	15 ± 1	70 ± 20		r2	0.54 ± 0.06	62 ± 13
Ro; 270 μg kg^{-1} min^{-1} + MDL 100907 (16.7; 0.83 μg kg^{-1} min^{-1})	5	0.44 ± 0.06	r1	0.77 ± 0.12	83 ± 29	9 ± 1	r1	13 ± 1	42 ± 12	0.21± 0.07	r1	0.36 ± 0.09	103 ± 42
			r2	0.72 ± 0.10	72 ± 22		r2	13 ± 2	42 ± 14		r2	0.29± 0.08	53 ± 37

Table 4.7b Ro 60-0175 + MDL 100907 (16.7; 0.83 μg kg^{-1} min^{-1}) i.v. experiments in urethane anaesthetized rats: baseline and test values and changes (Δ) in volume threshold, pressure threshold and residual volume. r1 and r2 represents reflex 1 and reflex 2.

Experimental group	n	EUS-EMG			Urethral Pressure (UP)			Mean Arterial Pressure (MAP)			Heart Rate (HR)		
		Baseline μV	Test μV	Δ %	Baseline mmHg	Test mmHg	Δ %	Baseline mmHg	Test mmHg	Δ bpm	Baseline bpm	Test bpm	Δ bpm
SB 242084 (22.5; 3.5 μg kg^{-1} min^{-1})	5	0.10 ± 0.02	0.10 ± 0.05	-	28 ± 1	27 ± 0.2	-4 ± 1	106 ± 1	102 ± 0.2	-4 ± 1	353 ± 2	357 ± 2	4 ± 3
SB 242084 (67.5; 3.5 μg kg^{-1} min^{-1})	5	0.10 ± 0.02	0.13 ± 0.05	-	28 ± 1	32 ± 0.1	9 ± 1	106 ± 1	88 ± 0.5	-17 ± 1	353 ± 2	326 ± 2	-26 ± 3

Table 4.8a SB 242084 (22.5; 3.5 and 67.5; 3.5 μg kg^{-1} min^{-1}) i.v. infusion experiments in urethane anaesthetized rats: baseline and test values and changes (Δ) in external sphincter (EUS) EMG, urethral pressure (UP), mean blood pressure (MAP) and heart rate (HR)

Experimental Group	n	Volume Threshold				Pressure Threshold				Residual Volume			
		Baseline ml		Test ml	Δ %	Baseline mmHg		Test mmHg	Δ %	Baseline ml		Test ml	Δ %
SB 242084 (22.5; 3.5 μg kg^{-1} min^{-1})	5	0.40 ± 0.07	r1	0.43 ± 0.08	6 ± 6	8 ± 1	r1	6 ± 1	-24 ± 11	0.17 ± 0.1	r1	0.21 ± 0.11	-1.2 ± 34
			r2	0.39 ± 0.08	-5.2 ± 6		r2	8 ± 2	-2 ± 19		r2	0.12 ± 0.04	-27 ± 27
SB 242084 (67.5; 3.5 μg kg^{-1} min^{-1})	5	0.40 ± 0.07	r1	0.43 ± 0.08	7 ± 13	8 ± 1	r1	7 ± 2	-12 ± 17	0.17 ± 0.1	r1	0.13 ± 0.06	-38 ± 23
			r2	0.37 ± 0.07	-7 ± 11		r2	7 ± 1	-14 ± 15		r2	0.14 ± 0.06	-29 ± 16

Table 4.8b SB 242084 (22.5; 3.5 and 67.5; 3.5 μg kg^{-1} min^{-1}) i.v. experiments in urethane anaesthetized rats: baseline and test values and changes (Δ) in volume threshold, pressure threshold and residual volume. r1 and r2 represents reflex 1 and reflex 2.

Experimental group	n	EUS-EMG			Urethral Pressure (UP)			Mean Arterial Pressure (MAP)			Heart Rate (HR)		
		Baseline μV	Test μV	Δ %	Baseline mmHg	Test mmHg	Δ %	Baseline mmHg	Test mmHg	Δ mmHg	Baseline bpm	Test bpm	Δ bpm
MDL 100907 (1.67; 0.83 μg kg^{-1} min^{-1})	5	0.48 ± 0.03	0.55 ± 0.04	-	26 ± 0.1	27 ± 0.1	4 ± 1	112 ± 1	100 ± 1	-11 ± 0.2	346 ± 2	357 ± 2	11 ± 3
MDL 100907 (5.01; 0.83 μg kg^{-1} min^{-1})	5	0.48 ± 0.03	0.35 ± 0.02	-	26 ± 0.1	28 ± 0.1	7 ± 1	112 ± 1	95 ± 1	-16 ± 2	346 ± 2	358 ± 2	13 ± 3
MDL 100907 (16.7; 0.83 μg kg^{-1} min^{-1})	5	0.48 ± 0.03	0.57 ± 0.02	-	26 ± 0.1	27 ± 0.1	4 ± 1	112 ± 1	87 ± 1	-25 ± 0.4	346 ± 2	389 ± 2	43 ± 2

Table 4.9a MDL 100907 (1.67; 0.83, 5.01; 0.83 and 16.7; 0.83 μg kg^{-1} min^{-1}) i.v. infusion experiments in urethane anaesthetized rats: baseline and test values and changes (Δ) in external sphincter (EUS) EMG, urethral pressure (UP), mean blood pressure (MAP) and heart rate (HR)

Experimental Group	n	Volume Threshold				Pressure Threshold				Residual Volume			
		Baseline ml		Test ml	Δ %	Baseline mmHg		Test mmHg	Δ %	Baseline ml		Test ml	Δ %
MDL 100907 (1.67; 0.83 μg kg^{-1} min^{-1})	5	0.35 ± 0.05	r1	0.46 ± 0.07	38 ± 10	8 ± 1	r1	8 ± 1	13 ± 18	0.10 ± 0.03	r1	0.13 ± 0.05	31 ± 25
			r2	0.45 ± 0.07	30 ± 11		r2	8 ± 1	10 ± 19		r2	0.12 ± 0.04	19 ± 30
MDL 100907 (5.01; 0.83 μg kg^{-1} min^{-1})	5	0.35 ± 0.05	r1	0.43 ± 0.11	16 ± 15	8 ± 1	r1	9 ± 2	17 ± 19	0.10 ± 0.03	r1	0.12 ± 0.05	5 ± 25
			r2	0.44 ± 0.07	30 ± 12		r2	9 ± 1	11 ± 14		r2	0.10 ± 0.05	43 ± 78
MDL 100907 (16.7; 0.83 μg kg^{-1} min^{-1})	5	0.35 ± 0.05	r1	0.44 ± 0.11	18 ± 15	8 ± 1	r1	8 ± 1	4 ± 22	0.10 ± 0.03	r1	0.16 ± 0.07	77 ± 75
			r2	0.41 ± 0.08	12 ± 14		r2	7 ± 1	-5 ± 16		r2	0.10 ± 0.02	-25 ± 11

Table 4.9b MDL 100907 (1.67; 0.83, 5.01; 0.83 and 16.7; 0.83 μg kg^{-1} min^{-1}) i.v. experiments in urethane anaesthetized rats: baseline and test values and changes (Δ) in volume threshold, pressure threshold and residual volume. r1 and r2 represents reflex 1 and reflex 2.

Chapter 5

Site of action of 5-HT_2 receptor agonists

5.1 Introduction

As mentioned in the previous chapters, both 5-HT_{2A} and 5-HT_{2C} receptors were observed to be involved in the control of micturition. However, the site of action of these two receptor subtypes was not established in the previous chapters. Several studies in the literature have demonstrated the involvement of central 5-HT_1 and 5-HT_7 receptors in the control of micturition although there is not much in the literature with regards to central 5-HT_2 receptors and their involvement in bladder and urethral function. A study by Espey *et al* (1992) provided a hint for the involvement of central 5-HT_2 receptors in micturition where they observed decreases in volume threshold following intrathecal administration of the 5-$HT_{1/2}$ receptor antagonist methysergide in awake cats. Additionally, there is no pharmacological evidence for the involvement of central 5-HT_2 receptors in smooth and striated urethral responses, although their involvement can be postulated from binding and/or molecular studies where 5-HT_2 receptor mRNA was identified in the sacral parasympathetic nucleus and the Onuf's nucleus of several species (Helton *et al.*, 1994). In addition to innervating the bladder, sacral parasympathetic nerves have been shown to be inhibitory on the urethra (de Groat *et al.*, 1993; Ralevic & Burnstock, 1998) whereas motorneurones arising from the Onuf's nucleus have been found to be either excitatory or inhibitory (see Fraser & Chancellor, 2003) on striated muscle activity.

It is clear from evidence in the literature that central 5-HT-pathways are involved in the control of micturition. The aim of the present study was therefore to investigate the site of action of the 5-HT_2 receptor agonist evoked responses observed on the bladder and

urethra in the previous chapters (chapters 3 and 4). 5-HT_2 receptor agonists were administered either supraspinally (i.c.v.) or spinally (i.t.), and their effects on the bladder and urethra recorded. In some experiments, rats were pretreated with the peripherally acting non-selective 5-HT_2 receptor antagonist BW 501C67 (Mawson & Whittington, 1970) in order to eliminate any peripheral actions of the 5-HT_2 receptor agonists on the bladder and/or the urethra.

5.2 Results

Baseline values for all variables are shown in Tables 5.3-5.5.

5.2.1 Vehicle Control

Administration of 5-HT_2 receptor agonist vehicle (0.9% wv^{-1} saline; i.v., i.c.v. and i.t.; n = 3-5) or 5-HT_2 receptor antagonist vehicle (100% DMSO; i.v; n = 3-5) evoked no significant changes in baseline EUS-EMG signal, urethral pressure and bladder distension caused by infusion of saline at a rate of 0.1 ml min^{-1} (micturition reflex). Baseline MAP and HR were also unaffected.

5.2.2 Effect of BW 501C67 a peripheral acting 5-HT receptor antagonist

BW 501C67 (1 mg kg^{-1}, i.v; n = 5) and saline had no significant effects on baseline EUS-EMG signal, urethral pressure, the micturition reflex, MAP and HR (Figure 5.1a & 5.2b).

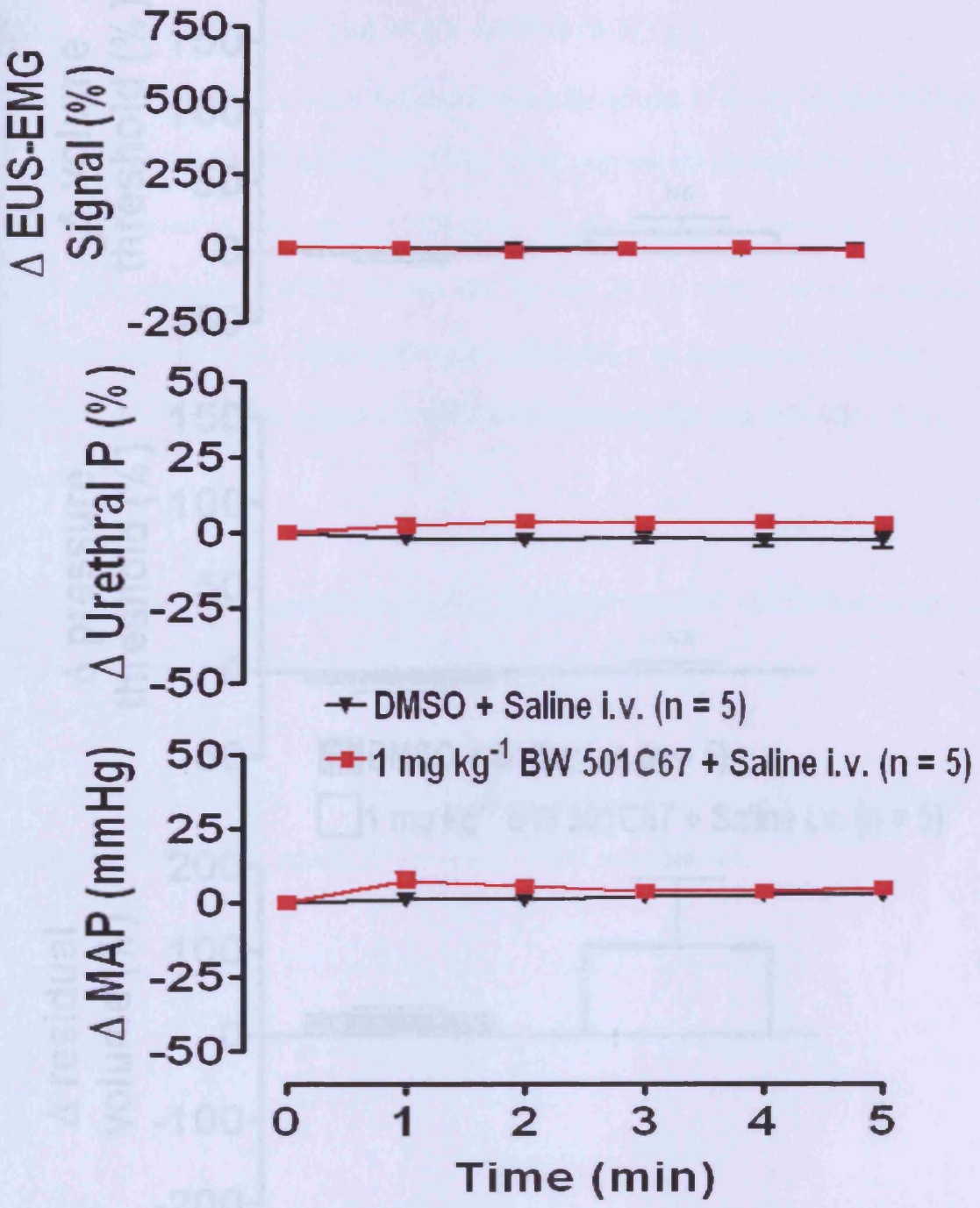

Figure 5.1a Urethane anaesthetised female rats: a comparison of the effects of BW 501C67 and vehicle (saline) on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (UP) and mean arterial blood pressure (MAP). Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by BW 501C67 and vehicle were compared with DMSO + saline control using two-way analysis of variance followed by the least significant difference test.

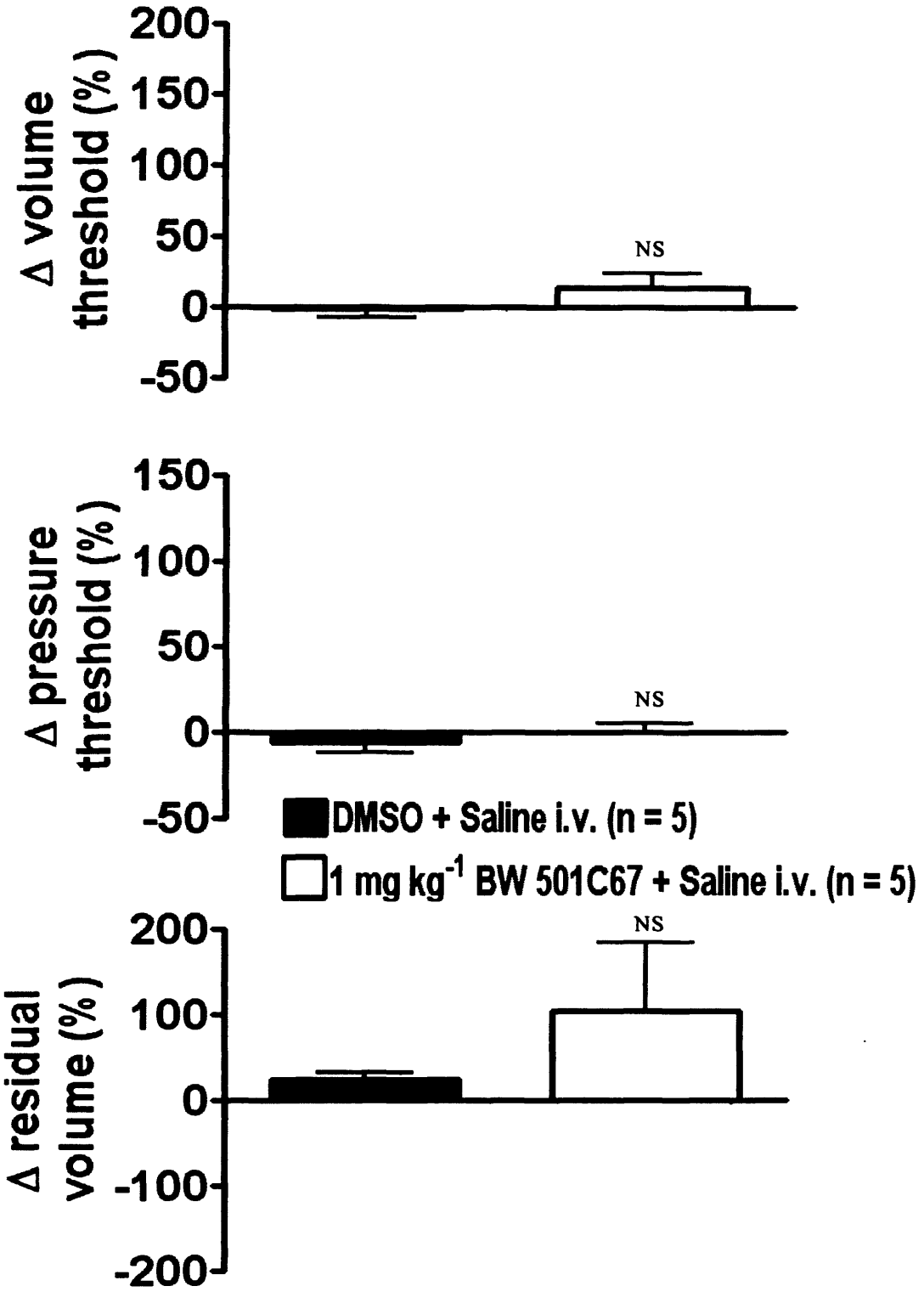

Figure 5.1b Urethane anaesthetised female rats: a comparison of the effects of BW 501C67 and vehicle (saline) on percentage changes (Δ) in volume threshold, pressure threshold and residual volume. Each bar represents mean value and vertical bars show the s.e.mean. Changes caused by BW 501C67 and saline were compared with DMSO + saline control using Student's unpaired t test. NS non-significant.

5.2.3 Effect of BW 501C67 on agonist responses

5.2.3.1 BW 501C67 and WAY 161503 (5-HT_{2C})

BW 501C67 (100 μg kg^{-1}, i.v; n = 5) failed to block the effects of WAY 161503 (300 μg kg^{-1}, i.v) on baseline EUS-EMG signal (273 ± 32%) and urethral pressure (5 ± 1%; Figure 5.2a). Onset of appearance of EUS-EMG firing following administration of WAY 161503 pre-treated with BW 501C67 was 82 ± 22s (c.f. 28 ± 11 WAY 161503 alone) and this evoked increase in EUS-EMG activity was observed to be ongoing up to 10 min, after which the bladder was emptied to test the micturition reflex and EUS-EMG firing stopped.

On the micturition reflex, pre-treatment of WAY 161503 with BW 501C67 had no effect on any of the variables measured (Figure 5.2b).

The pressor effect of WAY 161503 was significantly decreased following pre-treatment with BW 501C67 to 9 ± 1 mmHg (Figure 5.2a). HR was unaffected.

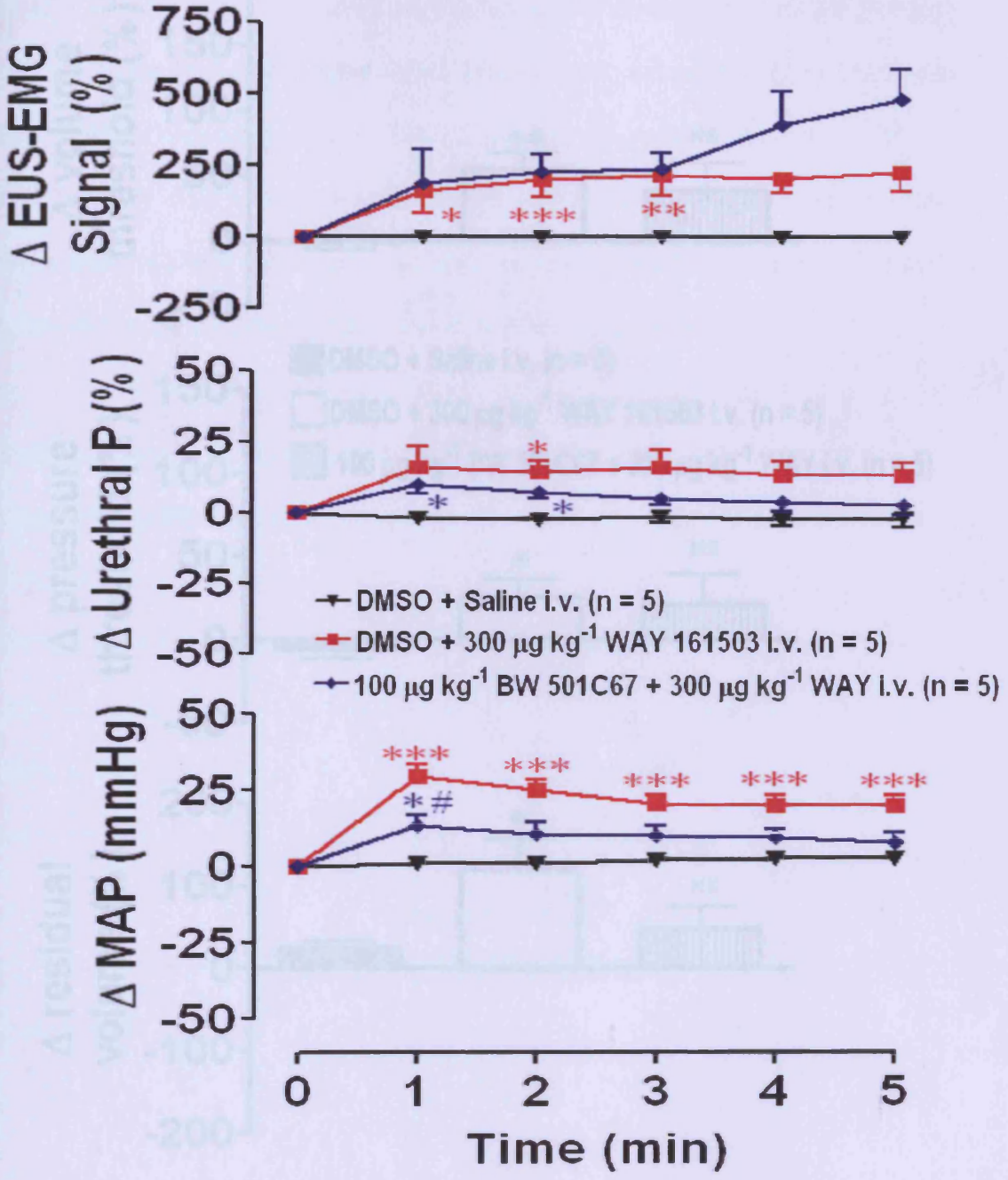

Figure 5.2a Urethane anaesthetised female rats: a comparison of the effects of vehicle (DMSO + saline) and pre-treatment of WAY 161503 with BW 501C67 on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (UP) and mean arterial blood pressure (MAP). Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by BW 501C67 and WAY 161503 were compared with DMSO + saline control using two-way analysis of variance followed by the least significant difference test. *,# $P<0.05$, ** $P<0.01$, *** $P<0.001$. (*), compared to DMSO + Saline, (#), compared to DMSO + WAY 161503.

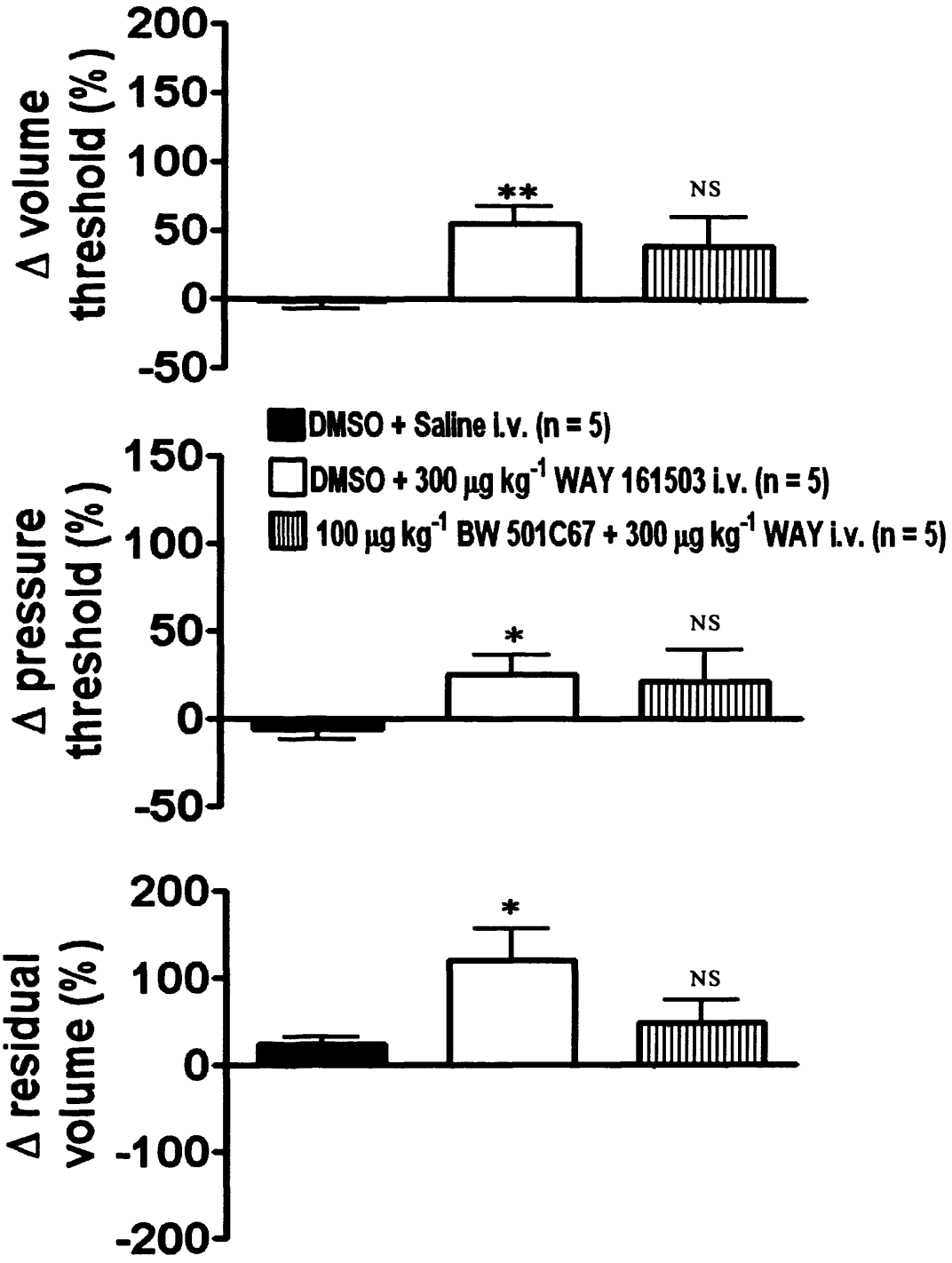

Figure 5.2b Urethane anaesthetised female rats: a comparison of the effects of vehicle (DMSO + saline) and pre-treatment of WAY 161503 with BW 501C67 on percentage changes (Δ) in volume threshold, pressure threshold and residual volume. Each bar represents mean value and vertical bars show the s.e.mean. Changes caused by BW 501C67 and WAY 161503 were compared with saline control using Student's unpaired t test.
* $P<0.05$, ** $P<0.01$, NS non-significant.

5.2.3.2 BW 501C67 and mCPP (5-HT_{2C})

BW 501C67 (100 μg kg^{-1}, i.v; n = 5) also failed to block the effects of mCPP (300 μg kg^{-1}) on baseline EUS-EMG signal (269 ± 44%) and urethral pressure (20 ± 2%; Figure 5.3a). Onset of appearance of EUS-EMG firing following administration of mCPP pre-treated with BW 501C67 was 16 ± 5s (c.f. 20 ± 5 mCPP alone). The increase in EUS-EMG activity was again observed to be ongoing up to 10 min after which the bladder was emptied to test the micturition reflex and EUS-EMG firing stopped.

In the presence of BW 501C67, mCPP still blocked the micturition reflex (as seen in chapter 3 for mCPP alone).

MAP and HR were again unaffected in these experiments.

5.2.3.3 BW 501C67 and DOI

BW 501C67 (100 μg kg^{-1} and 1 mg kg^{-1}, i.v; n = 4/5) failed to block the effects of DOI (100 μg kg^{-1}, i.v.) on baseline EUS-EMG signal (211 ± 29% and 133 ± 12%; Figure 5.4a). Urethral pressure was unaffected (Figure 5.4a). Onset of appearance of EUS-EMG firing following administration of DOI pre-treated with both doses of BW 501C67 was 22 ± 4s and 58 ± 20s respectively (c.f. 17 ± 3s DOI alone). The onset of appearance of EUS-EMG firing following pre-treatment of DOI with the high dose of BW 501C67 was found to be significantly increased when compared to DOI alone (see table 5.1 for summary of onset times of EUS-EMG activity). These evoked increases in EUS-EMG activity again were observed to be ongoing up to 10 min until the bladder was emptied to test the micturition reflex and EUS-EMG firing stopped.

On the micturition reflex, BW 501C67 (100 μg kg^{-1}) failed to block the excitatory effect of DOI on the reflex whereas the high dose (1 mg kg^{-1}) significantly reversed the DOI evoked decrease in volume threshold to an increase (23 ± 7%; Figure 5.4b).

The pressor effect of DOI was attenuated following pre-treatment with BW 501C67. HR was unaffected.

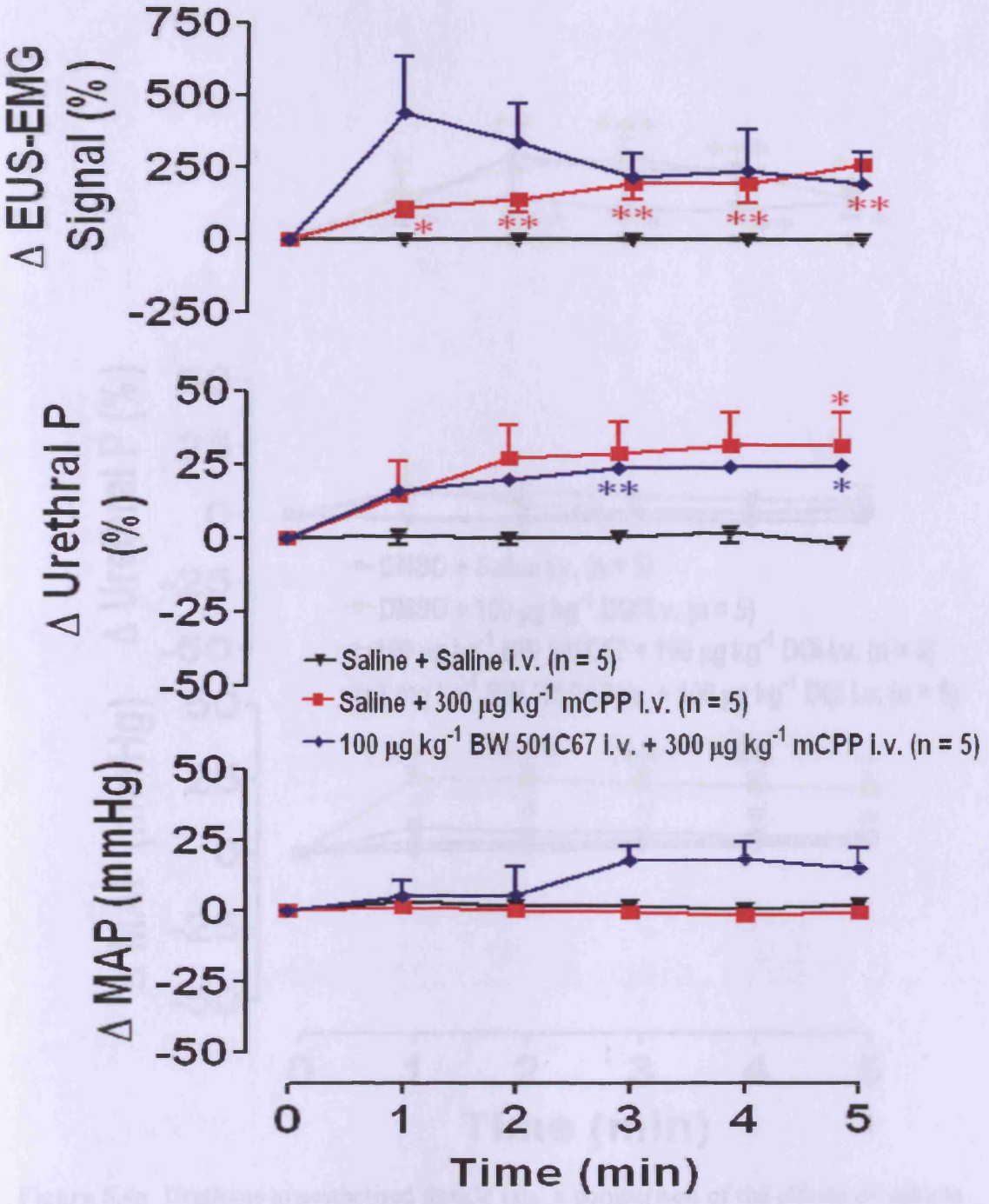

Figure 5.3a Urethane anaesthetised female rats: a comparison of the effects of vehicle (DMSO + saline) and pre-treatment of mCPP with BW 501C67 on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (UP) and mean arterial blood pressure (MAP). Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by BW 501C67 and mCPP were compared with saline + saline control using two-way analysis of variance followed by the least significant difference test. * $P<0.05$, ** $P<0.01$.

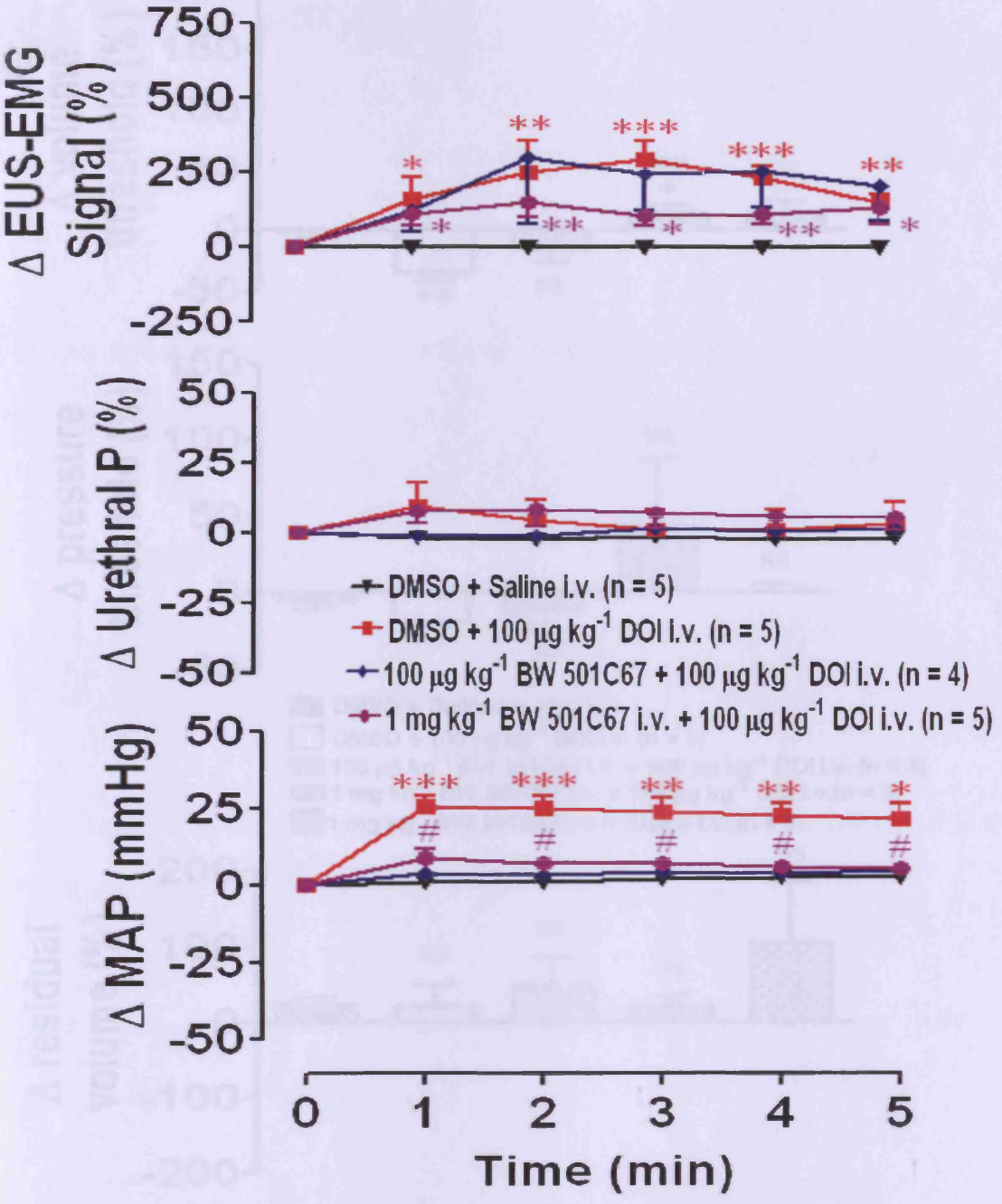

Figure 5.4a Urethane anaesthetised female rats: a comparison of the effects of vehicle (DMSO + saline) and pre-treatment of DOI with BW 501C67 on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (UP) and mean arterial blood pressure (MAP). Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by BW 501C67 and DOI were compared with DMSO + saline control using two-way analysis of variance followed by the least significant difference test. (*), compared to DMSO + Saline, (#), compared to DMSO + DOI.

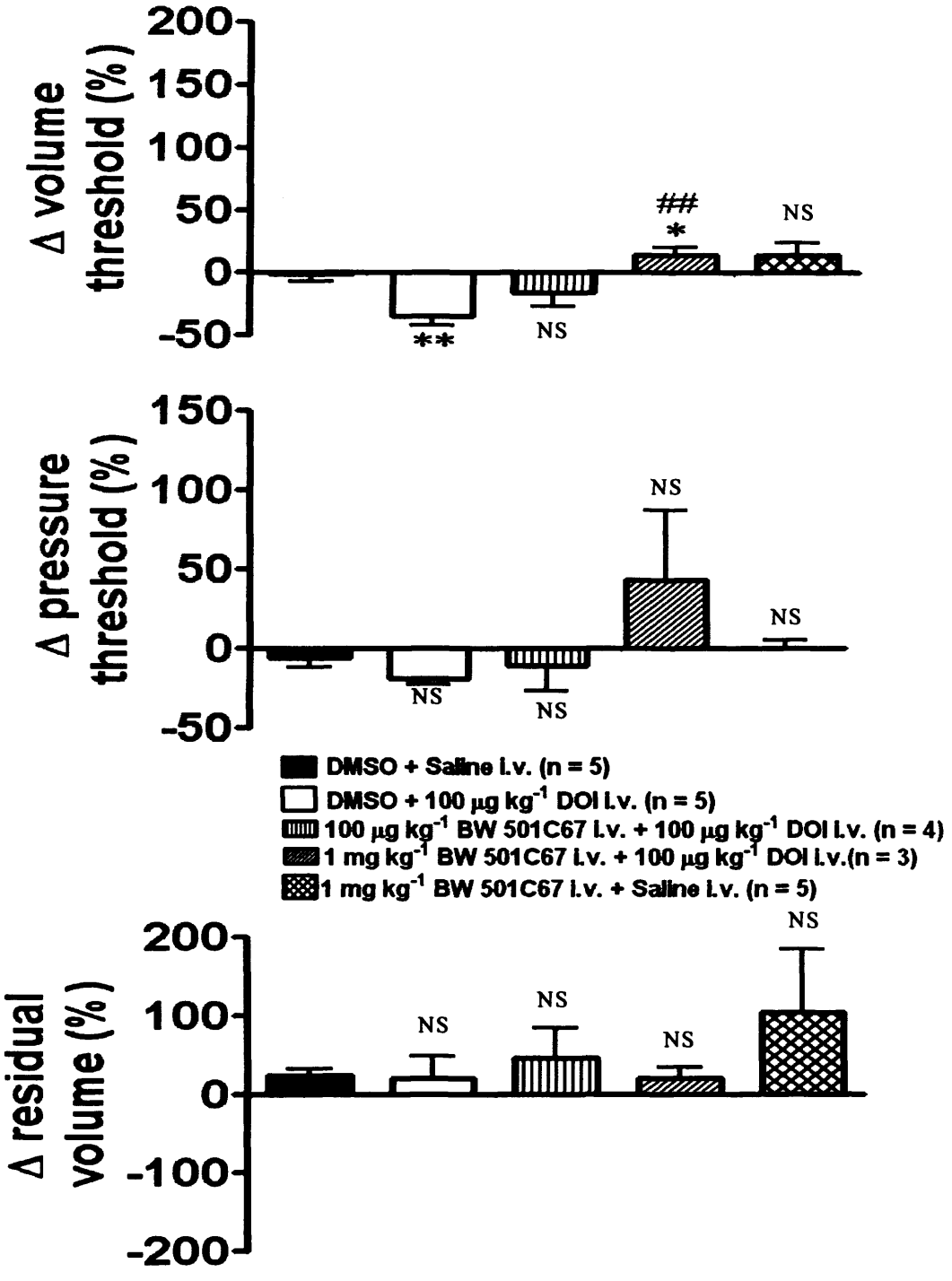

Figure 5.4b Urethane anaesthetised female rats: a comparison of the effects of vehicle (DMSO + saline) and pre-treatment of DOI with BW 501C67 on percentage changes (Δ) in volume threshold, pressure threshold and residual volume. Each bar represents mean value and vertical bars show the s.e.mean. Changes caused by BW 501C67 and DOI were compared with saline control using Student's unpaired t test. * $P<0.05$, **,## $P<0.01$, NS non-significant. (*), compared to DMSO + Saline, (#), compared to DMSO + DOI.

5.2.4 5-HT_2 receptor agonists i.c.v.

5.2.4.1 mCPP (5-HT_{2C})

mCPP i.c.v (300 μg kg^{-1}; 5 μl, n = 3) had no significant effect on baseline EUS-EMG signal, urethral pressure, MAP and HR when compared to saline control (Figure 5.5a & 5.5b).

On the micturition reflex, mCPP was inhibitory, with a significant increase observed on volume threshold (50 ± 11%; Figure 5.5c).

5.2.4.2 DOI (5-HT_{2A})

DOI i.c.v. (100 μg kg^{-1}; 5 μl, n = 5) had no significant effect on baseline EUS-EMG signal and urethral pressure when compared to saline control (Figure 5.6a & 5.6b).

On the micturition reflex, DOI evoked a significant increase in pressure threshold of 34 ± 9% (Figure & 5.6c).

DOI significantly increased MAP by 13 ± 2 mmHg. HR was unaffected (Figure 5.6b).

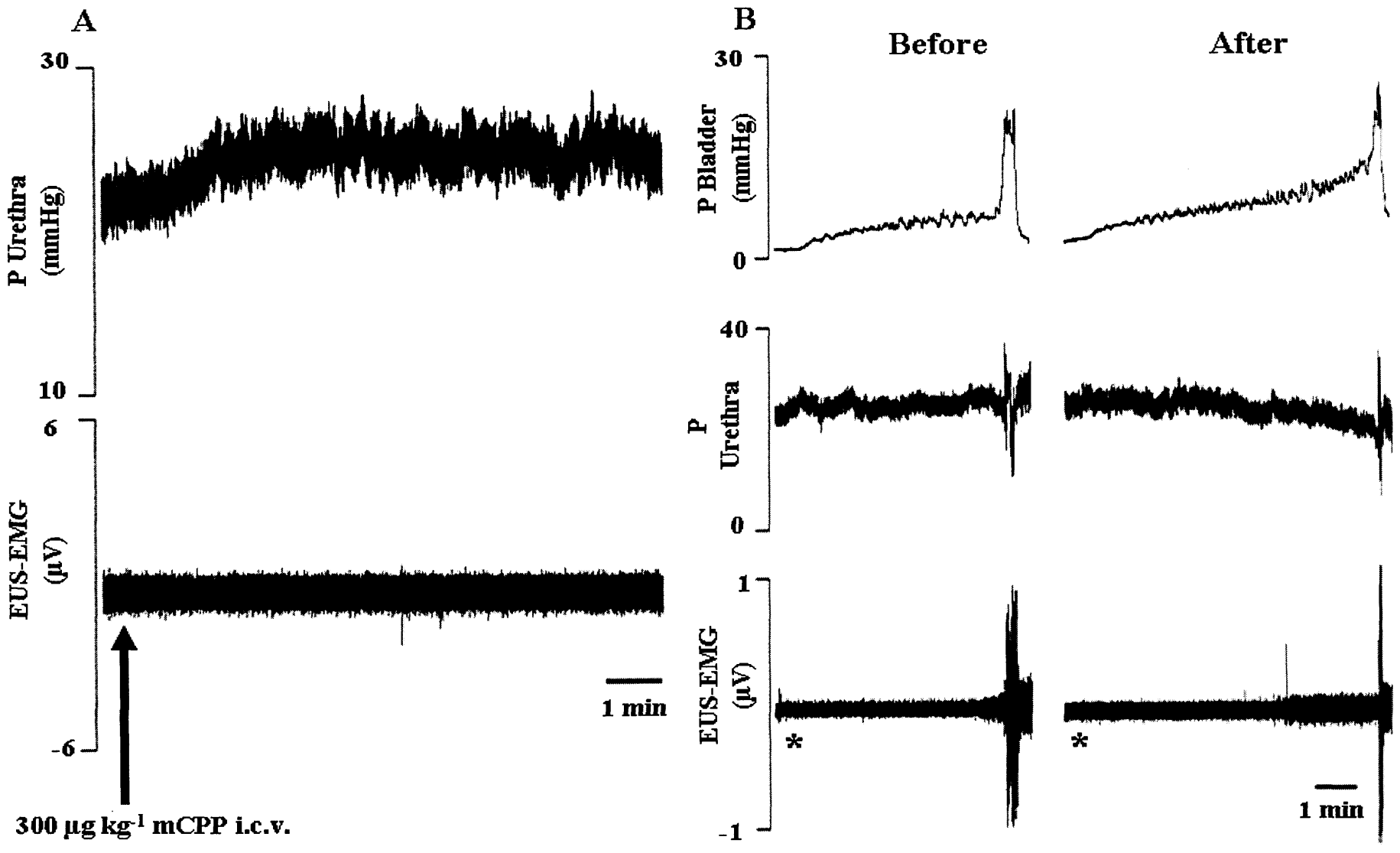

Figure 5.5a Traces showing the effects of mCPP (300 μg kg^{-1}, i.c.v.) on **A** baseline urethral pressure and EUS-EMG activity and **B** Δ in bladder and urethral pressures and raw urethral striated muscle. * denotes onset of saline infusion into the bladder.

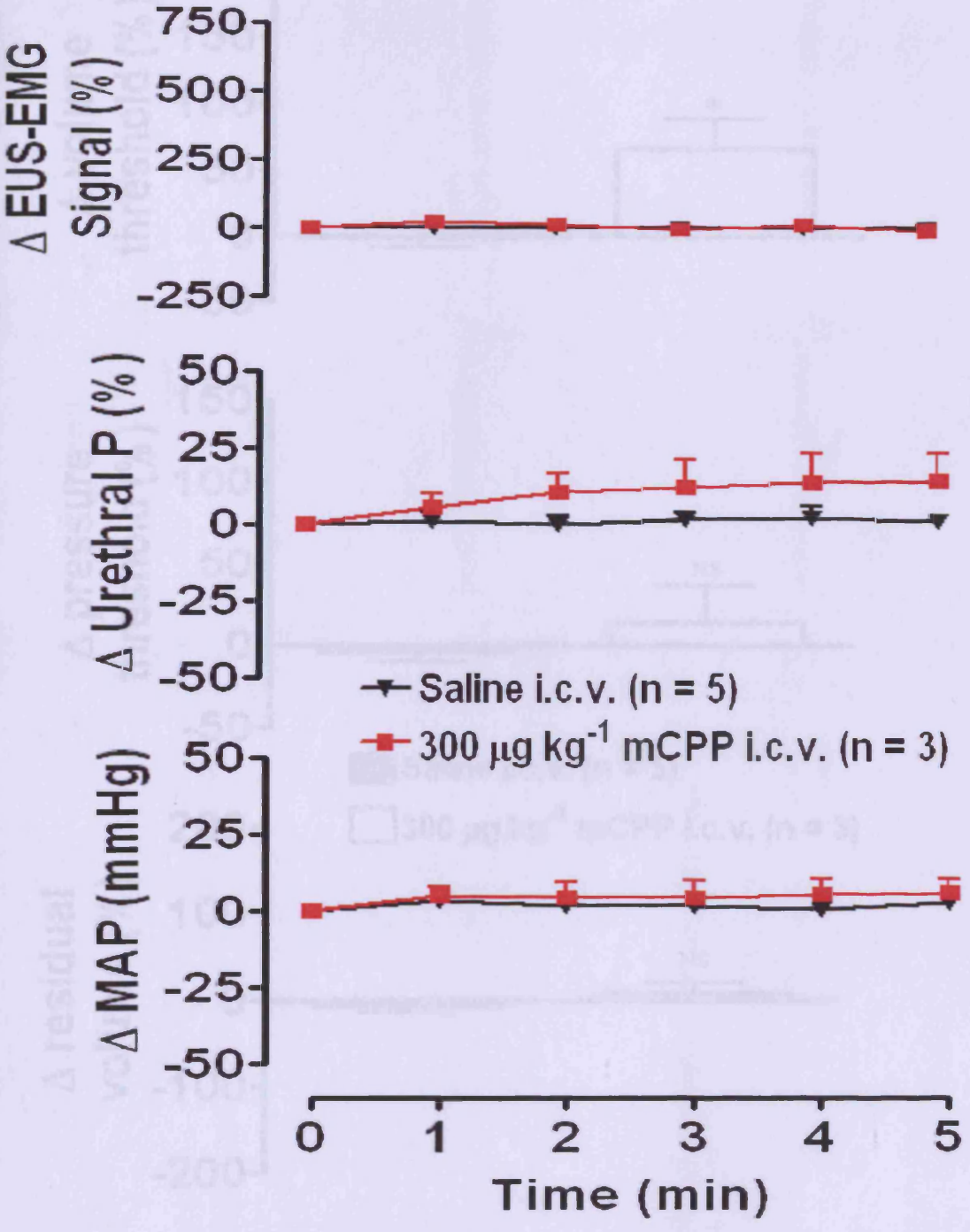

Figure 5.5b Urethane anaesthetised female rats: a comparison of the effects of i.c.v. administration of vehicle (saline) and mCPP on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (UP) and mean arterial blood pressure (MAP). Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by mCPP were compared with saline control using two-way analysis of variance followed by the least significant difference test.

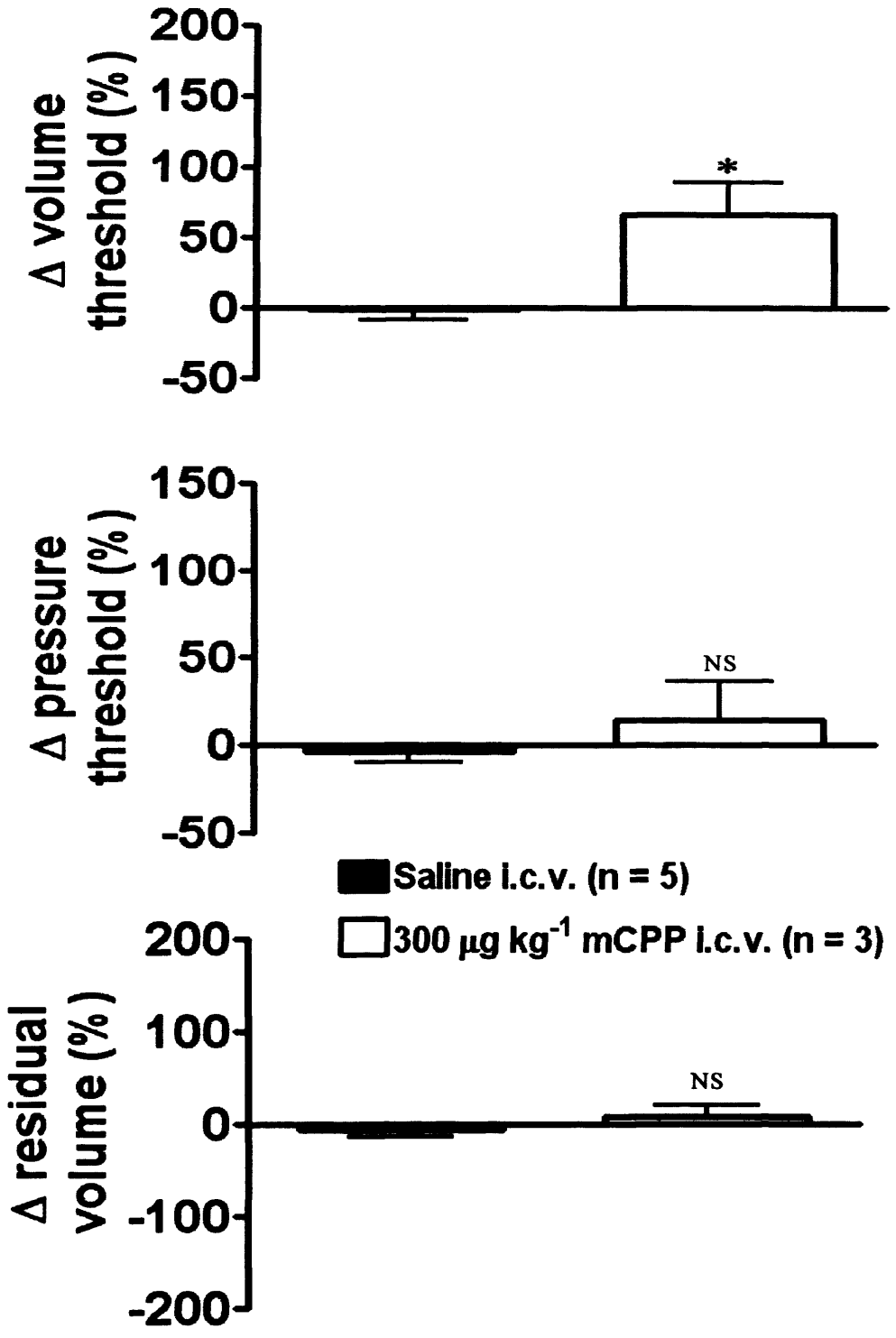

Figure 5.5c Urethane anaesthetised female rats: a comparison of the effects of i.c.v. administration of vehicle (saline) and mCPP on percentage changes (Δ) in volume threshold, pressure threshold and residual volume. Each bar represents mean value and vertical bars show the s.e.mean. Changes caused by mCPP were compared with saline control using Student's unpaired t test. * $P< 0.05$, NS non-significant.

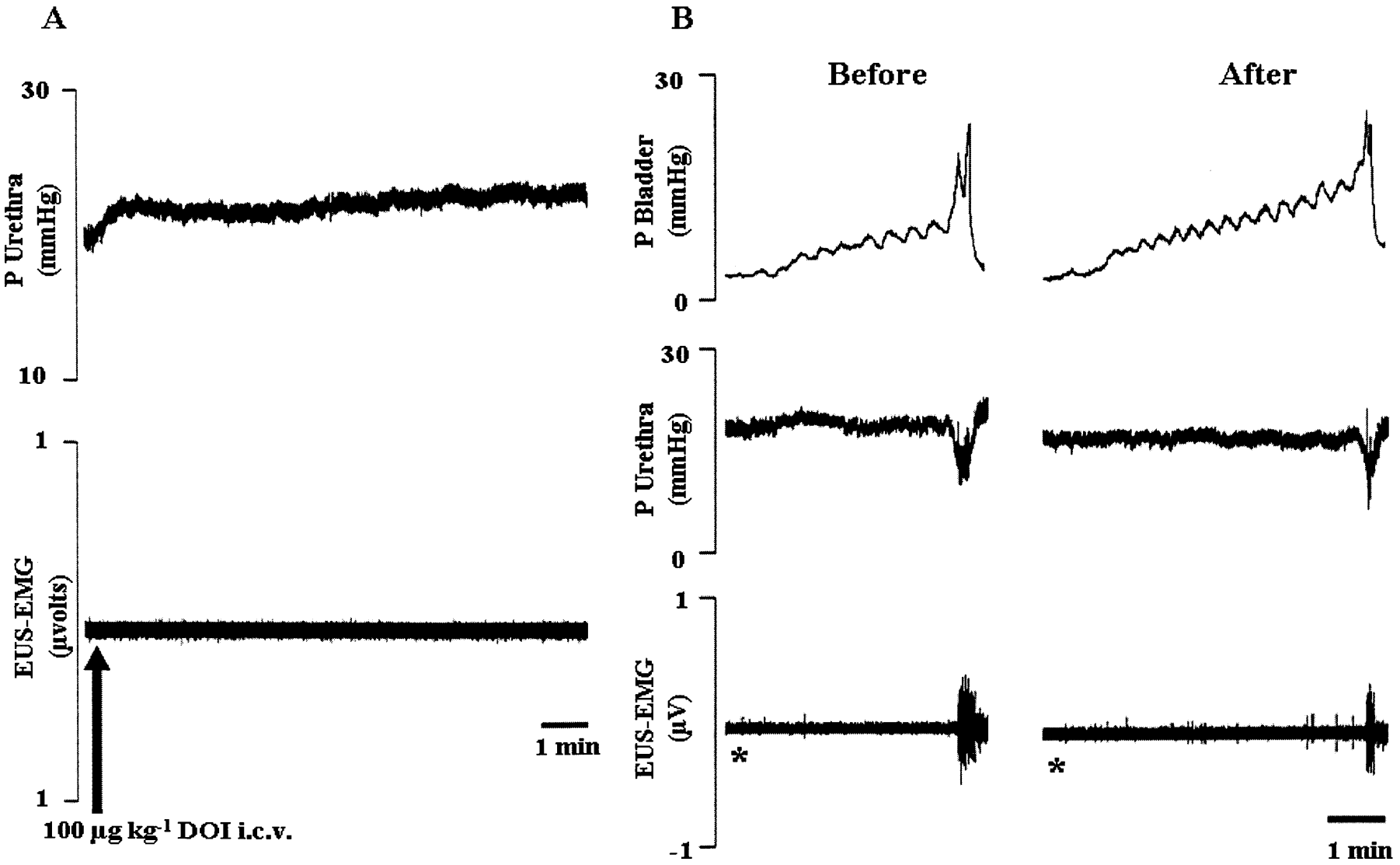

Figure 5.6a Traces showing the effects of DOI (100 µg kg^{-1}, i.c.v.) on **A** baseline urethral pressure and EUS-EMG activity and **B** Δ in bladder and urethral pressure and raw urethral striated muscle. * denotes onset of saline infusion into the bladder.

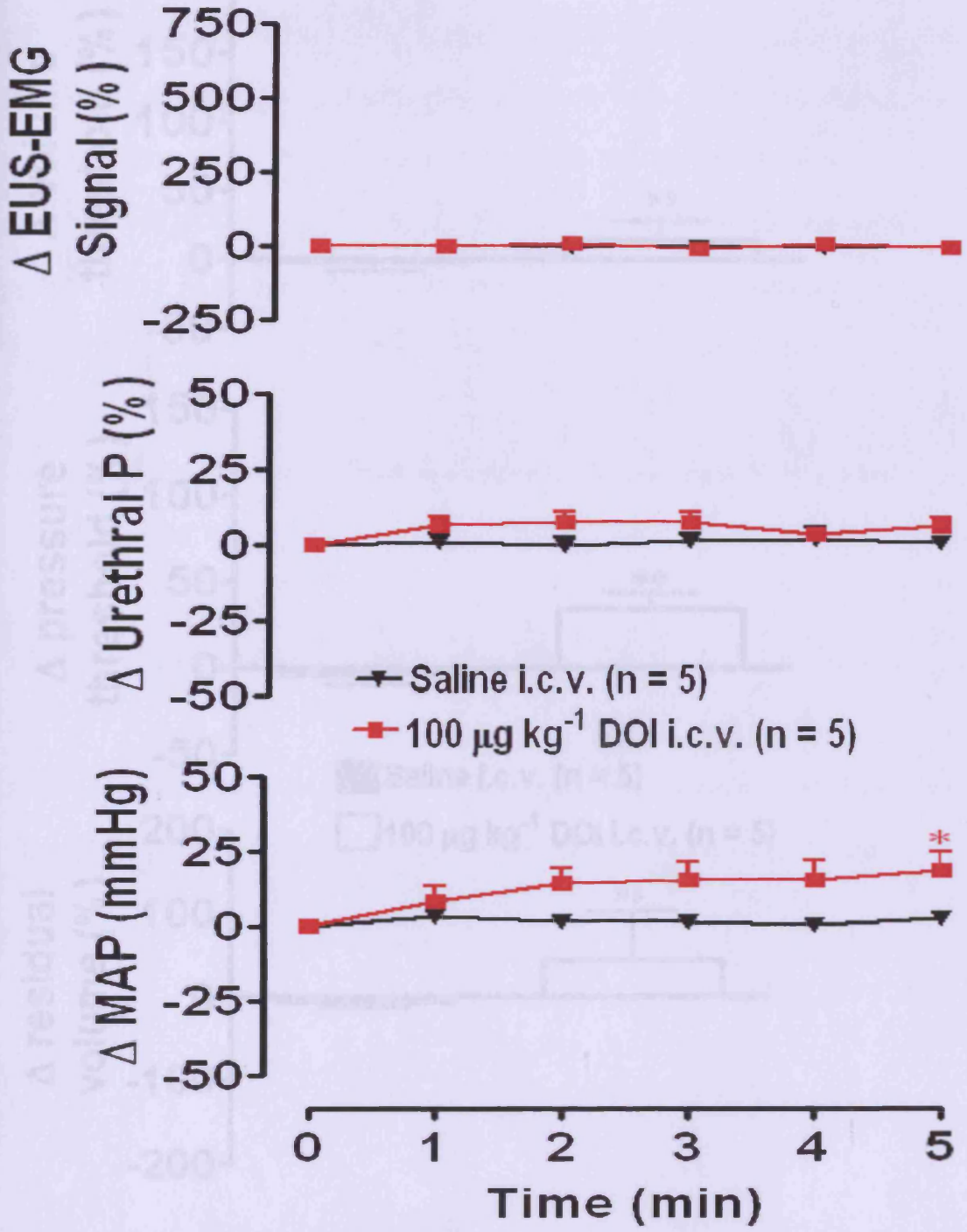

Figure 5.6b Urethane anaesthetised female rats: a comparison of the effects of i.c.v. administration of vehicle (saline) and DOI on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal, urethral pressure (UP) and mean arterial blood pressure (MAP). Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by DOI were compared with saline control using two-way analysis of variance followed by the least significant difference test. * $P<0.05$.

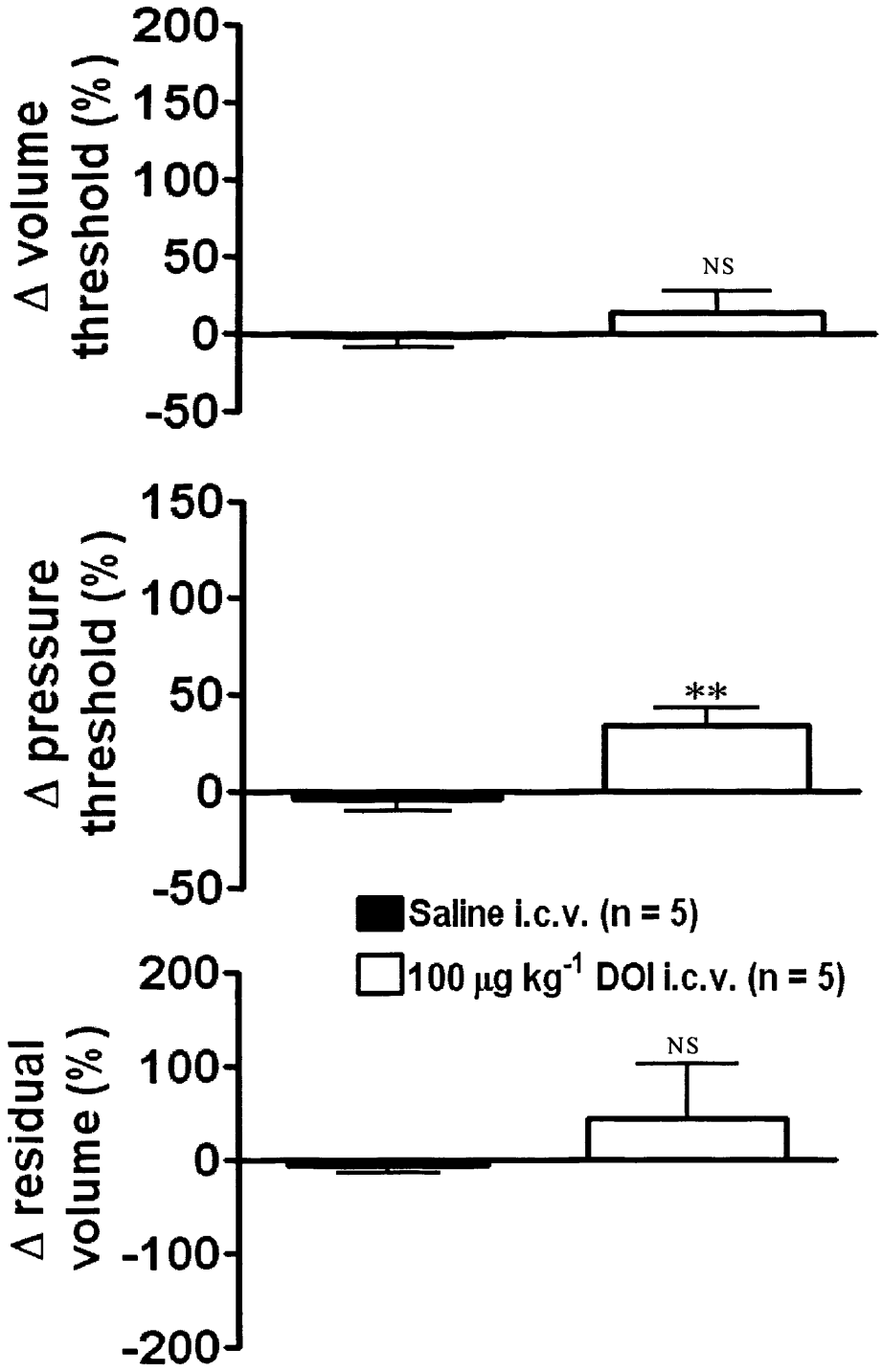

Figure 5.6c Urethane anaesthetised female rats: a comparison of the effects of i.c.v. administration of vehicle (saline) and DOI on percentage changes (Δ) in volume threshold, pressure threshold and residual volume. Each bar represents mean value and vertical bars show the s.e.mean. Changes caused by DOI were compared with saline control using Student's unpaired t test. ** $P< 0.05$, NS non-significant.

5.2.5 5-HT_2 receptor agonists i.t.

5.2.5.1 DOI (5-HT_{2A})

DOI (100 μg kg^{-1}; 10 μl, n = 3) significantly increased baseline EUS-EMG signal (24 ± 5%; Figure 5.7a & 5.7b). Onset of appearance of EUS-EMG firing following administration of DOI i.t. was 85 ± 68s (c.f. 17 ± 3s for i.v. DOI). Unlike the bolus i.v. data (see Chapter 3), the duration of EUS-EMG firing was shorter, lasting for approximately 3 min.

On the micturition reflex, i.t. DOI had no significant effect on any of the variables measured (Figure 5.7c).

DOI had no significant effect on either MAP or HR.

* Due to the positioning of the animal, the millar probe was not inserted into the urethral orifice and thus urethral pressure was not recorded for the intrathecal experiments.

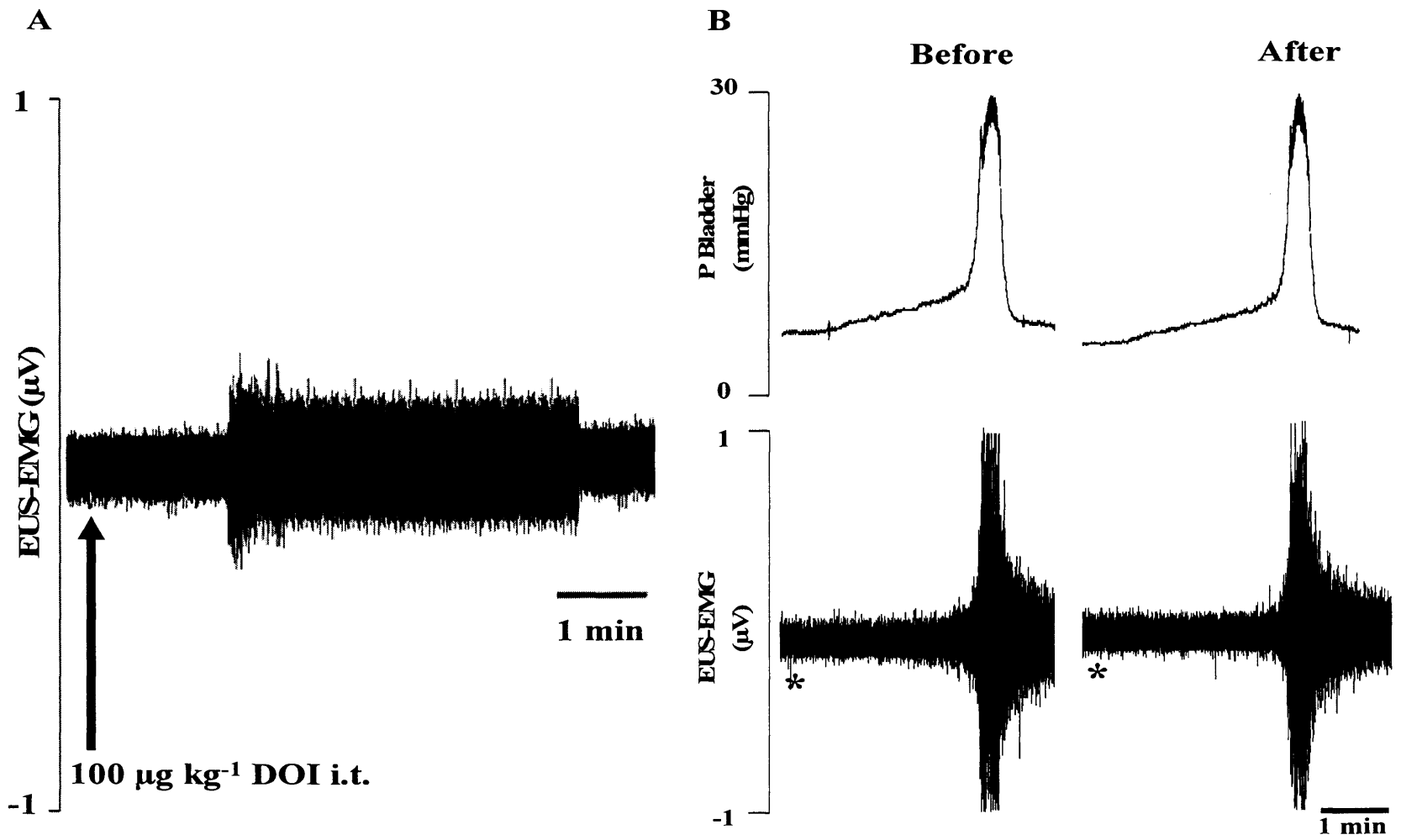

Figure 5.7a Traces showing the effects of DOI (100 μg kg^{-1}, i.t.) on **A** baseline EUS-EMG activity and **B** Δ in bladder pressure and raw urethral striated muscle. * denotes onset of saline infusion into the bladder.

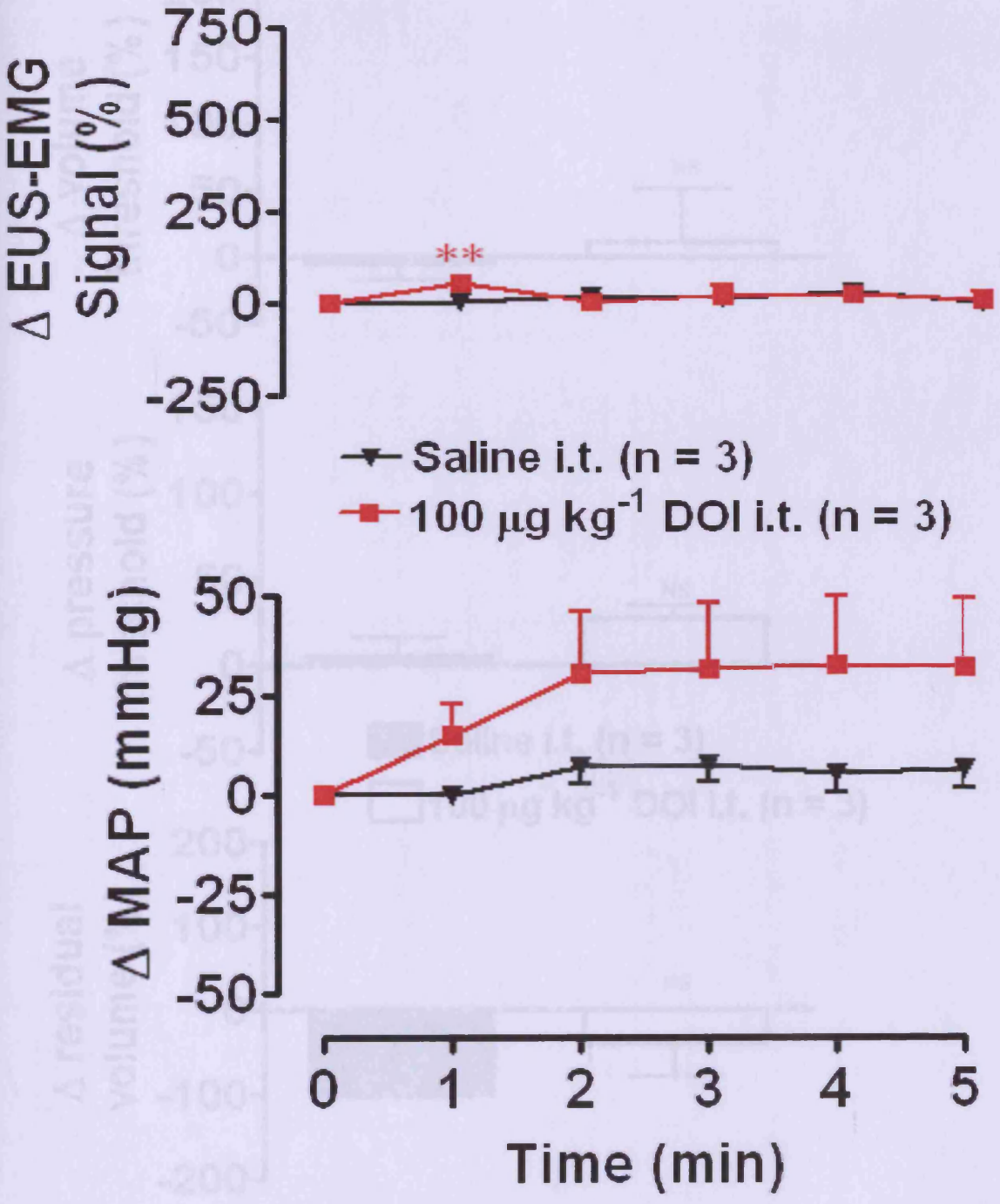

Figure 5.7b Urethane anaesthetised female rats: a comparison of the effects of i.t. administration of vehicle (saline) and DOI on percentage changes (Δ) in external urethral sphincter (EUS) EMG signal and mean arterial blood pressure (MAP). Each point represents the mean value and vertical bars show the s.e.mean. Changes caused by DOI were compared with saline control using two-way analysis of variance followed by the least significant difference test. ** $P<0.01$.

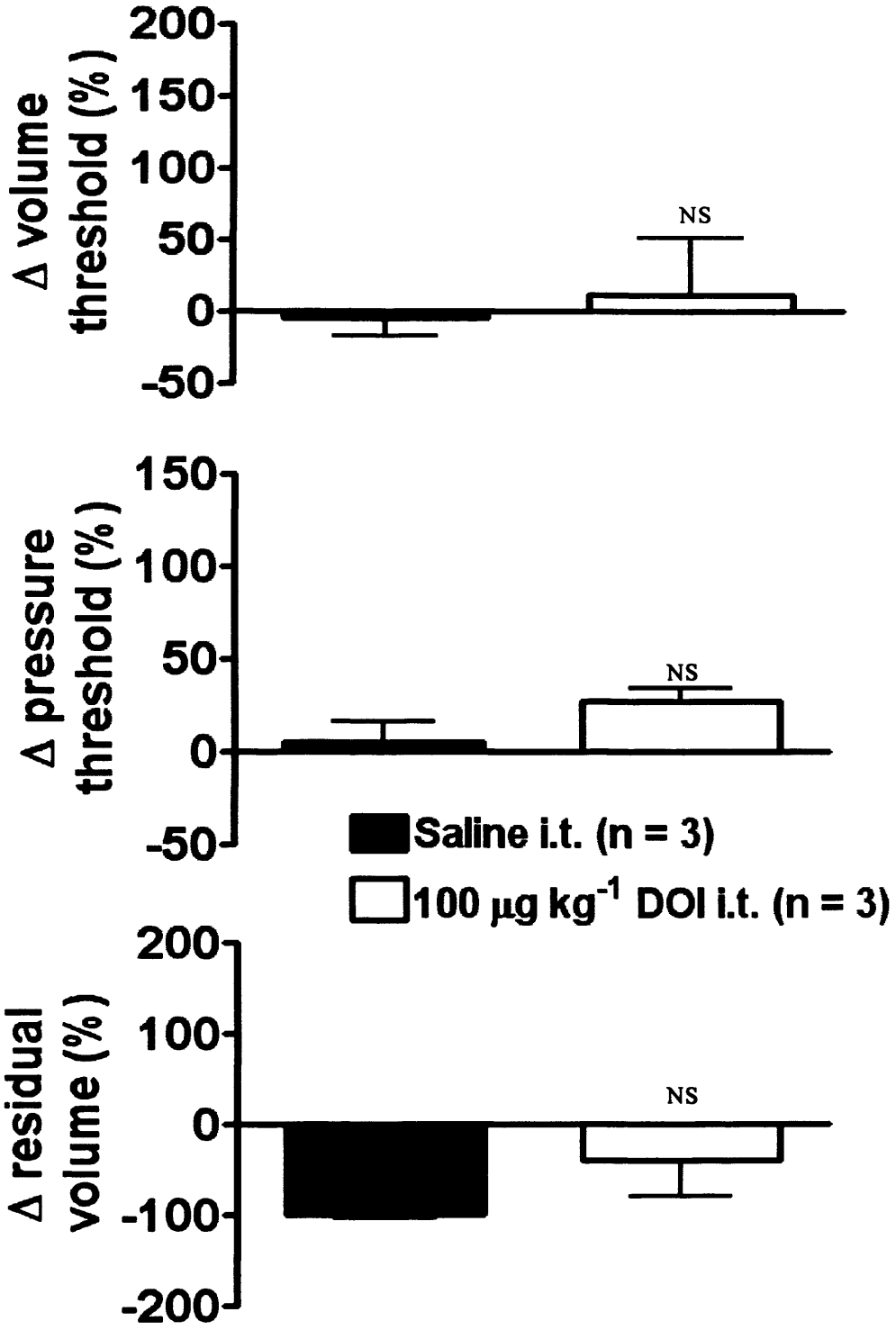

Figure 5.7c Urethane anaesthetised female rats: a comparison of the effects of i.t. administration of vehicle (saline) and DOI on percentage changes (Δ) in volume threshold, pressure threshold and residual volume. Each bar represents mean value and vertical bars show the s.e.mean. Changes caused by DOI were compared with saline control using Student's unpaired t test. NS non-significant.

Drug & treatment	Receptor selectivity		EUS EMG	Urethral pressure	Micturition Reflex				Cardiovascular effects	
					Overall	Vol T	Pressure T	Residual Vol	Blood Pressure	Heart Rate
BW 501C67 (i.v.)	5-HT_2	antagonist	↔	↔	↔	↔	↔	↔	↔	↔
WAY 161503 BW 501C67 (i.v.)	5-HT_{2C}		no block, tended to potentiate	↑	↔	↔	↔	↔	↓	↔
mCPP BW 501C67 (i.v.)	5-HT_{2C}		no block, tended to potentiate	↑↑	abolished	XXXX	XXXX	XXXX	↑ (variable)	↔
DOI BW 501C67 (100) BW 501C67(1000) (i.v.)	5-HT_{2A}		↔ ↓ (not significant)	↔ ↔	no effect inhibited	↔ ↑	↔ ↔	↔ ↔	blocked blocked	↔ ↔
mCPP (i.c.v.)			↔	↔	inhibited	↑	↔	↔	↔	↔
DOI (i.c.v.)			↔	↔	inhibited	↔	↑↑	↔	↑	↔
DOI (i.t.)			↑↑ (lasted ~ 3 min)	not measured	no effect	↔	↔	↔	↑ (variable)	↔

Table 5.1 Summary of 5-HT_2 receptor agonist and/or antagonist evoked responses (i.v., i.c.v. & i.t.) on EUS-EMG, urethral pressure, micturition reflex and cardiovascular effects

Experimental Group	n	Onset time of EUS-EMG activity (s)
100 μg kg^{-1} BW + 300 μg kg^{-1} WAY 161503 i.v.	5	82 ± 33
100 μg kg^{-1} BW + 300 μg kg^{-1} mCPP i.v.	5	16 ± 5
100 μg kg^{-1} BW + 100 μg kg^{-1} DOI i.v.	4	22 ± 4
1 mg kg^{-1} BW + 100 μg kg^{-1} DOI i.v.	3	* 58 ± 20

B

Experimental Group	n	Onset time of EUS-EMG activity (s)
100 μg kg^{-1} DOI i.t.	3	85 ± 68

Table 5.2 Summary of onset times of appearance of EUS-EMG activity following **A** pre-treatment of WAY 161503, mCPP and DOI with BW 501C67 (i.v.); **B** intrathecal DOI. All values are expressed as means ± sem.

* Onset of appearance of EUS-EMG activity following 1 mg kg^{-1} BW + 100 μg kg^{-1} DOI i.v. was found to be significantly ($P<0.05$) increased when compared to DOI alone (chapter 3).

5.3 Discussion

To determine whether the site of action of the 5-HT_{2C} receptor evoked responses on EUS-EMG activity, urethral pressure and the micturition reflex were mediated via a central or peripheral pathway, initial experiments in this study utilized the peripheral acting 5-HT_2 receptor antagonist BW 501C67. BW 501C67, although a potent and selective 5-HT_2 receptor antagonist, has been shown to be a poor blood-brain barrier penetrant (Mawson & Whittington, 1970), and is thus known to be a 'peripheral acting' 5-HT_2 receptor antagonist. A dose of 100 $\mu g\ kg^{-1}$ for BW 501C67 was chosen as it has been previously reported to be an effective dose at blocking peripheral responses to 5-HT without blocking serotonin-induced behavioural responses that are thought to be mediated centrally (Mawson & Whittington, 1970). Therefore in the present study, data with BW 501C67 indicates that the effect of mCPP, WAY 161503 and DOI on evoking EUS-EMG activity is due to a central action. Interestingly the low dose of BW 501C67 had a tendency to cause potentiation of the mCPP evoked EUS-EMG activity in the first minute, while for WAY 161503 this tendency was delayed. The tendency to cause this potentiation was not observed for DOI. DOI was also tested against a very high dose of BW 501C67, which also failed to block the evoked EUS-EMG activity, although there was a tendency to reduce the size of the evoked EUS-EMG signal. However, this reduction in size of the EUS-EMG signal was observed not to be significant when compared to DOI alone. The large dose of BW 501C67 also failed to affect baseline variables and the micturition reflex when administered on its own. Further, the increase in urethral pressure caused by both mCPP and WAY 161503 was unaffected as was the inhibitory action of mCPP and WAY 161503 on the micturition reflex by BW 501C67.

This indicates that 5-HT_{2C} receptors located centrally cause inhibition of the micturition reflex and is also consistent with the view that 5-HT_{2C} receptors have not been found outside the central nervous system. There is little data on the affinity of BW 501C67 for 5-HT_2 receptor subtypes; however Anderson *et al.*, (1992) did report that BW 501C67 had a pK_D of 9.5 at 5-HT_{2A} and a pK_D of 8.5 at 5-HT_{2C} receptors, although nothing was reported on BW 501C67 affinity for the 5-HT_{2B} receptor. Thus it is not possible to conclude whether the failure of BW 501C67 to interfere with the evoked increase in urethral function is due to this effect being a central and/or peripheral mediated action.

I.c.v. administration of mCPP failed to cause increases in urethral pressure or EUS-EMG activity. Additionally, mCPP given i.c.v. failed to completely block the micturition reflex as it did i.v. suggesting additional sites of action for 5-HT_{2C} receptor in the control of the micturition reflex. This could be at a sacral spinal level along with that site involved in evoking EUS-EMG activity. In this respect DOI given i.t. at the level of the sacral spinal cord did evoke EUS-EMG activity but failed to affect the micturition reflex suggesting that 5-HT_{2A} receptors involved in the control of micturition are at a supraspinal level. These data do not exclude 5-HT_{2C} receptors being involved in micturition at the supraspinal level and further implicate 5-HT_{2A} receptors activating the external urethral sphincter activity at the level of the spinal cord. In this respect, 5-HT_{2C} receptors have been localised in the parasympathetic nucleus of the sacral spinal cord (Bancillia *et al.*, 1999). The most obvious sites for the 5-HT_{2A} receptor involvement in EUS-EMG activation is at the sacral spinal cord where the Onuf's nucleus site is present and also where neurones innervating the external urethral sphincter which is composed of skeletal

muscle is found. The Onuf's nucleus also innervates the anal sphincter and has been found to contain a high density of 5-HT_{2A} and 5-ht_{5A} receptors (Doly *et al.*, 2004 a,b) as well as 5-HT_{2C} receptors (Bancillia *et al.*, 1999). As the bladder is required to be 80% full before this effect of 5-HT_{2A} receptor agonists can be observed, this would imply that a level of afferent input from the bladder to these motorneurones is required and thus these receptors could be on the afferent terminals increasing the release of the afferent transmitter. However the motorneurones in most cases have the 5-HT_{2A} receptor located postsynaptically (Doly *et al.*, 2004b) and this has been recently confirmed for Onuf's nucleus which also receives 5-HT innervation (Xu *et al.* 2007). Further, the present study does not indicate that the 5-HT_{2A} receptor plays a physiological role in the control of the EUS nor does the present data indicate a physiological role for 5-HT_{2C} receptors in the micturition reflex. However, it is possible that these motorneurones in the Onuf's nucleus have to be slightly depolarised by a certain level of afferent excitation before activation of 5-HT_{2A} receptors can evoke action potentials. Additionally, motorneurones innervating the EUS did not exhibit 5-HT_{2A} receptor labelling (Xu *et al.*, 2007) and in female rats the density of 5-HT_{2A} receptors was observed to be much lower than in males indicating that they are more involved in ejaculatory and erectile function (Xu *et al.*, 2007). It should be further considered that particular 5-HT_{2A} receptors involved in EUS-EMG activation could be located in another area or there may be, intriguingly, a cross over in pharmacology between 5-HT_{2A} and 5-ht_{5A} receptors. In this respect the 5-HT_{2A} receptor antagonist ritanserin does have good affinity for this receptor, however little is know about the other ligands (see Thomas, 2006), although mianserin has a pKi of 7.1 (Dr David Thomas personal communication).

The BW 501C67 studies indicate that the effects of WAY 161503 i.v. on the micturition reflex have a peripheral component as the changes caused by WAY 161503 were not significantly different from control after BW 501C67 pretreatment, although the ability for mCPP to block the reflex was unaffected. For DOI, the ability to decrease volume threshold was blocked and reversed to an increase by BW 501C67, again indicating that the excitatory effect of DOI could be due to a peripheral action involving to a degree increased bladder smooth muscle tone through 5-HT_{2A} receptor activation. However, i.c.v. DOI also caused a decrease in the pressure but not the volume threshold suggesting a central site of action. It is therefore possible that the DOI leaked out of the brain to have a peripheral action, although peripherally DOI was observed to only affect volume threshold. These DOI actions on the micturition reflex are intriguing. Indeed it has been established that an inhibitory action on serotonergic neurones in the dorsal raphe nucleus is synonymous to excitatory actions on the micturition reflex (Testa *et al.*, 2001). A study by Boothman *et al* (2003) found that systemic administration of DOI inhibited 5-HT cell firing and these effects were observed to be reversed following pre-treatment with the 5-HT_{2A} receptor antagonist MDL 100907, thus confirming the inhibition of 5-HT cell firing by the 5-HT_{2A} receptor subtype. It can therefore be hypothesized that DOI administered i.v. inhibits 5-HT cell firing in the dorsal raphe neurones which in turn decreases spinal 5-HT and removes the endogenous inhibitory mechanism responsible for inhibiting the micturition reflex and thus the excitatory effects observed on the micturition reflex. This would imply that there is a central component by which 5-HT_{2A} receptors mediate their actions on the micturition reflex. However, from the present data,

it is still difficult to conclude how much of the effects of DOI on the micturition reflex are due to a peripheral and or central site of action.

In the guinea-pig EUS excitation has been demonstrated to be due to activation of 5-HT_{2C} rather than 5-HT_{2A} receptors (McMurray & Miner, 2005). However the function of the external sphincter in this species is similar to that of man but not the rat (see McMurray *et al.*, 2006), where it is part of the "guarding reflex" i.e. EUS-EMG activity increases as the bladder fills to prevent incontinence whereas in the rat the external sphincter has pulsatile activity during micturition. This pulsatile activity is meant to aid voiding by acting as a pump. Therefore, as mentioned previously in the rat the external sphincter can be considered to aid micturition whereas in the guinea-pigs it can be considered to be part of the mechanism where urine is retained i.e. inhibitory to micturition, which seems to be the general role of 5-HT_{2C} receptors in micturition.

The peripheral acting 5-HT_2 receptor antagonist BW 501C67 was observed to cause significant decreases in the pressor response of both the 5-HT_{2C} and 5-HT_{2A} receptor agonists WAY 161503 and DOI respectively. Additionally, administration of DOI i.c.v. and not i.t. significantly increased blood pressure. Neither of the compounds administered i.v. or i.c.v. were observed to have significant effects on heart rate. The present data would therefore suggest that the pressor responses observed following administration of both the 5-HT_{2C} and 5-HT_{2A} receptor agonists are due to either a central or peripheral mechanism. Moreover, only supraspinal and not spinal 5-HT_{2A} receptors are involved in mediating the pressor response observed. Due to concerns on the

selectivity of the compounds utilized in this study, WAY 161503 i.v. was probably activating 5-HT_{2A} in addition to 5-HT_{2C} receptors, and the pressor response observed was probably due to activation of peripheral 5-HT_{2A} receptors. Indeed the pressor response of WAY 161503 was blocked following pre-treatment with BW 501C67 thus confirming a peripheral mechanism. 5-HT_{2A} and not 5-HT_{2C} receptors have been found to be widespread in the cardiovascular system with a predominant location in the arterial smooth muscle (Ullmer *et al.*, 1995). With regards to a peripheral mechanism as mentioned previously, it is possible that this 5-HT_{2A} receptor mediated effect on blood pressure occurs due to vascular smooth muscle contraction or central sympathoexcitation. 5-HT_{2A} receptors are also known to regulate vasopressin release which causes peripheral vasoconstriction thus resulting in a rise in blood pressure (Anderson *et al.*, 1992; Knowles & Ramage, 1999; Pérgola *et al.*, 1998). With regards to 5-HT_{2A} receptors acting centrally to mediate their actions on blood pressure, results in the present study are in agreement with previous cat data (Andersson *et al.*, 1995) where i.c.v. administration of DOI caused significant increases in blood pressure, heart rate, cardiac and splanchnic sympathetic nerve activity and a decrease in femoral arterial conductance. It can be postulated that DOI administered i.c.v. mediated its actions by activating a central angiotensinergic pathway which causes vasopressin release (Saydoff *et al.*, 1996) thus contracting vascular smooth muscle and causing an increase in blood pressure. Furthermore, as peripheral 5-HT_2 receptors were not blocked prior to administering DOI i.c.v., it may also be possible that the pressor response observed occurs via a peripheral mechanism.

5.4 Conclusion

In conclusion, the results from the present study demonstrate that activation of 5-HT_{2A} receptors at the level of sacral spinal cord is responsible for excitation of the EUS, and further supports the view that the inhibitory action of 5-HT_{2C} receptors on micturition is centrally mediated. Additionally, data from the present study also provides evidence for the involvement of both central and peripheral 5-HT_{2A} receptors in mediating blood pressure increases.

Experimental group	n	EUS-EMG				Urethral Pressure (UP)			Mean Arterial Pressure (MAP)			Heart Rate (HR)		
		Baseline μV	Test μV	Δ %	Onset s	Baseline mmHg	Test mmHg	Δ %	Baseline mmHg	Test mmHg	Δ	Baseline bpm	Test bpm	Δ
DMSO + Saline	5	0.26 ± 0.04	0.26 ± 0.02	-	-	22 ± 2	22 ± 2	-	125 ± 3	125 ± 3	-	329 ± 12	336 ± 2	-
1 mg kg^{-1} BW + Saline	5	0.46 ± 0.11	0.44 ± 0.02	-	-	23 ± 2	23 ± 0.4	3 ± 0.5	122 ± 3	126 ± 1	5 ± 1	352 ± 10	344 ± 3	-6 ± 1
100 μg kg^{-1} BW + 300 μg kg^{-1} WAY	5	0.36 ± 0.12	1.65 ± 0.23	273 ± 32	82 ± 22	22 ± 1	23 ± 0.23	5 ± 1	120 ± 4	127 ± 1	9 ± 1	371 ± 13	376 ± 2	3 ± 1
100 μg kg^{-1} BW + 300 μg kg^{-1} mCPP	5	0.74 ± 0.31	3.60 ± 0.60	269 ± 44	16 ± 5	19 ± 2	22 ± 1	20 ± 2	114 ± 5	124 ± 3	10 ± 2	340 ±13	317 ± 5	-10 ± 4
100 μg kg^{-1} BW + 100 μg kg^{-1} DOI	4	0.35 ± 0.06	1.00 ± 0.10	211 ± 29	22 ± 4	18 ± 2	18 ± 0.3	0.3 ± 0.4	108 ± 4	112 ± 1	4 ± 0.4	319 ± 23	303 ± 8	-13 ± 4
1 mg kg^{-1} BW + 100 μg kg^{-1} DOI	3	0.44 ± 0.08	1.00 ± 0.10	133 ± 12	58 ± 20	24 ± 2	26 ± 0.5	5 ± 1	124 ± 6	129 ± 1	5 ± 1	361 ± 9	364 ± 3	2 ± 1

Table 5.3a Peripheral acting 5-HT_2 receptor antagonist BW 501C67 on agonist responses i.v. experiments in urethane anaesthetized rats: baseline and test values and changes (Δ) in external sphincter (EUS) EMG, urethral pressure (UP), mean blood pressure (MAP) and heart rate (HR)

Experimental Group		Volume threshold			Pressure Threshold			Residual Volume		
		Baseline ml	Test ml	Δ %	Baseline mmHg	Test mmHg	Δ %	Baseline %	Test %	Δ %
DMSO + Saline	5	0.42 ± 0.06	0.42 ± 0.06	-	9 ± 1	9 ± 1	-	43 ± 9	43 ± 9	-
1 mg kg^{-1} BW + Saline	5	0.40 ± 0.06	0.45 ± 0.06	14 ± 10	8 ± 1	8 ± 1	1 ± 4	45 ± 14	54 ± 9	105 ± 79
100 µg kg^{-1} BW + 300 µg kg^{-1} WAY	5	0.32 ± 0.07	0.39 ± 0.02	39 ± 20	8 ± 0.4	10 ± 2	21 ± 18	26 ± 8	33 ± 6	48 ± 26
100 µg kg^{-1} BW + 300 µg kg^{-1} mCPP	5	-	-	-	-	-	-	-	-	-
100 µg kg^{-1} BW + 100 µg kg^{-1} DOI	4	0.27 ± 0.06	0.34 ± 0.06	-16 ± 12	11 ± 1	7 ± 1	-11 ± 17	32 ± 6	26 ± 7	46 ± 39
1 mg kg^{-1} BW + 100 µg kg^{-1} DOI	3	0.44 ± 0.15	0.57 ± 0.22	23 ± 7	9 ± 1	12 ± 2	44 ± 43	64 ± 6	76 ± 6	21 ± 13

Table 5.3b Peripheral acting 5-HT_2 receptor antagonist BW 501C67 on agonist responses i.v. experiments in urethane anaesthetized rats: baseline and test values and changes (Δ) volume threshold, pressure threshold and residual volume

(A)

Experimental group	n	EUS-EMG				Urethral Pressure (UP)			Mean Arterial Pressure (MAP)			Heart Rate (HR)		
		Baseline μV	Test μV	Δ %	Onset s	Baseline mmHg	Test mmHg	Δ %	Baseline mmHg	Test mmHg	Δ	Baseline bpm	Test bpm	Δ
Saline	5	0.32 ± 0.06	0.32 ± 0.02	-	-	19 ± 2	19 ± 0.1	1 ± 0.2	136 ± 5	137 ± 0.4	1 ± 0.4	324 ± 31	333 ± 2	8 ± 2
300 μg kg^{-1} mCPP	3	0.50 ± 0.12	0.50 ± 0.01	-	-	24 ± 4	24 ± 4	10 ± 1	130 ± 2	134 ± 1	5 ± 1	311 ± 6	283 ± 4	-28 ± 4
100 μg kg^{-1} DOI	5	0.54 ± 0.12	0.52 ± 0.01	-	-	21 ± 2	21 ± 2	6 ± 1	116 ± 2	129 ± 2	13 ± 2	358 ± 14	363 ± 1	5 ± 1

(B)

Experimental Group		Volume threshold			Pressure Threshold			Residual Volume		
		Baseline ml	Test ml	Δ %	Baseline mmHg	Test mmHg	Δ %	Baseline %	Test %	Δ %
Saline	5	0.27 ± 0.01	0.26 ± 0.05	-1 ± 8	9 ± 1	8 ± 1	-4 ± 6	58 ± 10	55 ± 11	-5 ± 8
300 μg kg^{-1} mCPP	3	0.43 ± 0.04	0.64 ± 0.06	50 ± 11	8 ± 1	9 ± 1	14 ± 23	50 ± 21	59 ± 26	8 ± 12
100 μg kg^{-1} DOI	5	0.33 ± 0.05	0.39 ± 0.07	14 ± 13	8 ± 1	11 ± 1	34 ± 9	47 ± 8	49 ± 8	44 ± 58

Table 5.4a & 5.4b 5-HT_2 receptor agonist i.c.v. experiments in urethane anaesthetized rats: baseline and test values and changes (Δ) in **(A)** external sphincter (EUS) EMG, urethral pressure (UP), mean blood pressure (MAP) and heart rate (HR) and **(B)** volume threshold, pressure threshold and residual volume.

(A)

Experimental group	n	EUS-EMG				Urethral Pressure (UP)			Mean Arterial Pressure (MAP)			Heart Rate (HR)		
		Baseline μV	Test μV	Δ %	Onset s	Baseline mmHg	Test mmHg	Δ %	Baseline mmHg	Test mmHg	Δ	Baseline bpm	Test bpm	Δ
Saline	3	0.30 ± 0.01	0.38 ± 0.02	17 ± 2	-	-	-	-	108 ± 2	109 ± 0.3	1 ± 0.3	337 ± 10	339 ± 1	2 ± 1
100 μg kg^{-1} DOI	3	0.34 ± 0.07	0.54 ± 0.05	23 ± 5	142 ± 106	-	-	-	103 ± 9	129 ± 3	25 ± 3	342 ± 21	319 ± 6	-23 ± 6

(B)

Experimental Group		Volume threshold			Pressure Threshold			Residual Volume		
		Baseline ml	Test ml	Δ %	Baseline mmHg	Test mmHg	Δ %	Baseline %	Test %	Δ %
Saline	3	0.22 ± 0.01	0.26 ± 0.05	-4 ± 13	12 ± 1	13 ± 2	5 ± 11	37 ± 12	18 ± 5	-100 ± 4
100 μg kg^{-1} DOI	3	0.25 ± 0.01	0.39 ± 0.11	11 ± 40	13 ± 1	18 ± 2	27 ± 7	43 ± 9	39 ± 14	-39 ± 40

Table 5.5a & 5.5b 5-HT_2 receptor agonist i.t. experiments in urethane anaesthetized rats: baseline and test values and changes (Δ) in **(A)** external sphincter (EUS) EMG, urethral pressure (UP), mean blood pressure (MAP) and heart rate (HR) and **(B)** volume threshold, pressure threshold and residual volume.

Chapter 6

General Discussion

6 General Discussion

The present study investigated the role and sites of action of 5-HT_2 receptors in the control of micturition in urethane anaesthetized female rats. From the literature, 5-HT_2 receptor activation has been observed to inhibit micturition (Steers & de Groat, 1989, Guarneri *et al*, 1996) with the receptor proposed to mediate its actions either spinally, supraspinally or at both levels. Additionally, it has been hypothesized that 5-HT_{2C} receptors are involved in the supraspinal tonically active 5-HT_{1A} autoreceptor pathway responsible for the excitatory actions of the 5-HT_{1A} receptor where activation of 5-HT_{1A} autoreceptors reduces spinal 5-HT, which in turn reduces activation of the 5-HT_{2C} receptors that are known to be inhibitory on the micturition reflex (de Groat, 2002).

Experiments in the present study demonstrate the involvement of all three 5-HT_2 receptors in the control of micturition in urethane anaesthetized female rats. By utilizing commercially available 5-HT_2 receptor agonists and antagonists, it was established that all three receptor subtypes played a different role in the control of the lower urinary tract. The 5-HT_{2A} receptor subtype was observed to be excitatory on both the micturition reflex and external urethral sphincter activity and the 5-HT_{2B} receptor was observed to be involved in urethral smooth muscle contraction. Additionally, the 5-HT_{2C} receptor was found to be inhibitory on the micturition reflex. Both 5-HT_{2C} and 5-HT_{2A} receptors were observed to mediate their actions on the bladder and urethra via a central mechanism although a peripheral site of action on the urethra cannot be ruled out.

Attempting to deduce a mechanism by which the 5-HT_{2A} receptor mediated its actions on the external urethral sphincter was complicated further by the fact that recent molecular evidence has revealed labelling of the 5-ht_{5A} and not 5-HT_{2A} receptor on motorneurones directly innervating the external urethral sphincter (Xu *et al.*, 2007). It is therefore possible that both these receptors are involved in causing the observed increases in EUS-EMG activity via a synergistic pathway or some form of cross over in pharmacology takes place. Indeed both receptors are known to activate adenylyl cyclase (Garnovskaya *et al.*, 1995; Berg *et al.*, 1994; Francken *et al.*, 1998, 2001; Thomas *et al.*, 2004) thus providing further evidence for a possible interaction between these two receptor subtypes. Additionally, the results in this study provide evidence for the existence of species variation as the 5-HT_{2C} receptor was observed to be the main 5-HT_2 receptor subtype mediating increases in EUS-EMG activity and inhibiting the micturition reflex in the guinea pig (McMurray & Miner, 2005), whereas in the rat the 5-HT_{2A} receptor increased EUS-EMG activity and facilitated the micturition reflex. Moreover, in the dog, activation of the 5-HT_{2C} receptor was observed to increase urethral pressure (Conlon *et al.*, 2005), whereas in the present study, the 5-HT_{2B} receptor was revealed as the receptor responsible for increasing urethral pressure in the rat.

It is of interest from the present pharmacological experiments that the two urethral muscles function via independent receptors/mechanisms even though they have both been implicated as playing a role in continence in the rat (see Fraser & Chancellor, 2003). Interestingly, there has been a hint of the involvement of the external urethral sphincter in facilitating efficient voiding in the rat (Walters *et al.*, 2006). Indeed whilst the 5-HT_{2A}

receptor has been established as the main 5-HT_2 receptor involved in mediating the increases observed in external urethral sphincter activity, the 5-HT_{2B} receptor has been implicated to be involved in urethral smooth muscle contraction. Although structurally linked, the origin of activity of both urethral muscles has been observed to be different. The excitatory pathway responsible for urethral smooth muscle contraction originates in sympathetic preganglionic neurones with efferent input to the urethral smooth muscle mediated via the hypogastric nerves. On the other hand, the excitatory pathway responsible for external urethral sphincter activity originates from the Onuf's nucleus with efferent input to the external urethral sphincter mediated via the pudendal nerves. Moreover, tracer studies have revealed a different supraspinal circuitry controlling both urethral muscles. Pseudorabies virus injected into the urethral smooth muscle infected the pontine micturition centre which is known to be responsible for micturition whereas injecting the virus into the external urethral sphincter primarily infected the pontine storage center which directly projects to the Onuf's nucleus and plays a role in storage (see Fraser & Chancellor, 2003). However, to a lesser extent the pontine micturition center was also infected following injections into the external urethral sphincter thus suggesting a crossover may exist between the functions of the urethral striated muscle in both continence and micturition. Collectively with support from the literature findings described above, pharmacological evidence from the present study in the rat favours the role of the 5-HT_{2A} receptor in aiding micturition rather than continence, whereas 5-HT_{2B} and 5-HT_{2C} receptors are shown to be involved in continence. In theory, for effective continence to occur, an increase in external urethral sphincter activity should be synonymous to inhibition of the bladder as well as urethral smooth muscle contraction. It

has been proposed that for this to occur, afferent signals travel from the contracting external urethral sphincter (characterized by the increased EUS-EMG activity) via the pudendal nerves to the sacral spinal cord inhibit parasympathetic bladder motorneurones directly through spinal interneurones, as well as increasing sympathetic influence upon the bladder and urethral smooth muscle (see Park *et al.*, 1997). However, this does not appear to be the case in the rat from the present data, as an increase in EUS-EMG activity following activation of the 5-HT_{2A} receptor was characterized by excitatory actions on the bladder with no effects observed on the urethral smooth muscle thus providing evidence for a favourable action of the 5-HT_{2A} receptor in micturition. It would thus appear that afferent input from the external urethral sphincter to the sacral spinal cord activates parasympathetic bladder motorneurones as well as inhibiting the sympathetic influence to the bladder and urethral smooth muscle, with excitatory actions observed on the micturition reflex.

The inhibitory and excitatory actions of the 5-HT_2 receptor ligands on the micturition reflex in the present study is intriguing as it provides further evidence for the involvement of this receptor family on bladder function. Previous rat studies have revealed that activation of the 5-HT_{2C} receptor has an inhibitory action on micturition and somewhat surprising, antagonists to this receptor are without effect (Steers & de Groat, 1989; Guarneri *et al.*, 1996; Testa *et al.*, 2001). Moreover the present study also provides evidence for the excitatory actions of the 5-HT_{2A} receptor in bladder function in the rat. It has also been demonstrated that the effects observed on bladder function are mediated via central 5-HT_2 receptors as the peripheral acting 5-HT_2 receptor BW 501C67 failed to

attenuate/block the actions of either the 5-HT_{2C} or 5-HT_{2A} receptor agonists. Additionally, efficacy on the micturition reflex was observed following central administration of either 5-HT_{2C} or 5-HT_{2A} receptor agonists. However, from the present study there is no evidence for a physiological role of these receptors in micturition as slow infusions of the antagonists SB 242084 and MDL 100907 failed to reveal efficacy on the micturition reflex (see chapter 4). The lack of a physiological involvement of 5-HT_{2C} receptors in micturition is interesting as evidence from the present study disagrees with the supraspinal tonically active 5-HT_{1A} autoreceptor (negative feedback) pathway proposed by de Groat (2002) thought to involve the 5-HT_{2C} receptor.

In the present study, a problematic aspect of studying the function of 5-HT_2 receptor subtypes *in vivo* was the seemingly poor selectivity of the agonists used to study these receptors. Indeed from previous studies the so called 5-HT_{2C} receptor agonists WAY 161503, Ro 60-0175 and mCPP have displayed behavioural characteristics typical of the 5-HT_{2C} and not 5-$HT_{2A/2B}$ receptor including hypoactivity, penile grooming and hypophagia (Millan *et al.*, 1997; Martin *et al.*, 1998; Kennett *et al.*, 2000) with these effects reversed by selective 5-HT_{2C} receptor antagonists. The fact that the EUS-EMG increases observed in chapter 3 following bolus dosing of these agonists is blocked by both 5-HT_{2C} and 5-HT_{2A} receptor antagonists implied activation of both receptor subtypes, thus making interpretation of the data more difficult. Additionally the lack of blockade of the 5-HT_{2C} receptor evoked increase in urethral pressure laid further claim to a lack of selectivity of these ligands as this response was concluded to be 5-HT_{2B} receptor mediated. Furthermore, the commercially available agonists used to study the effects of

both the 5-HT_{2A} and 5-HT_{2B} receptors, DOI and BW 723C86 were reported as possessing very low selectivity for their respective receptor subtype and should thus be referred to as 5-$HT_{2A/2C}$ "preferring" rather than "selective" receptor agonists. Surprisingly, the selective 5-HT_{2C} receptor antagonist RS 102221 was observed to have inconsistent actions on 5-HT_{2C} receptor agonist evoked responses on the bladder and urethra thus adding more doubt as to the selectivity of these compounds *in vivo*. To overcome this problem, experiments in chapter 4 were designed to utilize a dosing regimen which involved infusion of the compound at a specific rate and dosage targeting the specific receptor subtype, with measures of free plasma concentration of the compound further confirming selectivity for the receptor subtype. By carrying out these controlled set of experiments, conclusions regarding the role played by the 5-HT_2 receptor family in the control of micturition could thus be made.

Although cardiovascular regulation was not the major focus of this thesis, the 5-HT_2 receptor was observed to be involved in cardiovascular regulation with the involvement of both central and peripheral receptors. Central and peripheral activation of 5-HT_{2A} receptors increased blood pressure and this was proposed to occur following the release of vasopressin which is known to cause vasoconstriction. Moreover, data from the present study was consistent with the view that tonic activation of 5-HT_{2B} receptors decreased blood pressure (see Ramage, 2001) with a mechanism thought to involve the release of nitric oxide which causes vascular relaxation. Finally, 5-HT_{2C} receptors were found not to be involved in cardiovascular regulation.

The experimental design and animal model used in these studies enabled further characterization of the involvement of 5-HT receptor subtypes in the control of micturition with a focus on the 5-HT_2 receptor family. The cystometry model used in the present study is a straightforward model of bladder and urethral functionality that enables acquisition of appropriate and relevant information regarding the effects of the compounds which can thereafter be translated into understanding autonomic innervation of the lower urinary tract. The female rat was chosen as the species of choice for the present study due to its ready availability within the academic and industrial laboratory. Additionally, the female rat has been extensively used to investigate the physiology and pathophysiology of the bladder and how this relates to the control of micturition. Furthermore, even though distinct differences exist between the two species, several similarities regarding voiding such as patterns of flow and peak bladder pressure (Walters *et al.*, 2006) exist, thus making the rat a suitable species to study lower urinary tract function.

The cystometric parameters measured on the micturition reflex in the present study included volume threshold, pressure threshold and residual volume. Justification for measuring these three parameters was to investigate the role played by 5-HT_2 receptors on voiding function. Data from the present study demonstrated that 5-HT_{2C} receptors affected the sensory limb of the micturition reflex pathway due to the increases observed on volume and pressure threshold, as well as maintaining bladder compliance. Additionally, it is also possible that this receptor subtype had an effect on the somatic efferent pathway due to the increase in residual volume which would suggest urethral

interference. On the other hand, the facilitatory actions of the 5-HT_{2A} receptors characterised by decreased volume threshold and altered pressure threshold (primarily an increase in pressure threshold) indicated a change had occurred in the bladder's ability to expand in order to accommodate urine (bladder compliance).

Future work in continuing with these studies would be to use the same compounds and translate them to a conscious rodent cystometry model which would be useful in enabling deductions be made on whether the actions mediated by the 5-HT_2 receptors in the anaesthetized animal are similar in the fully conscious state when the animal is undertaking everyday activities. Supraspinal and spinal electrophysiological recordings in combination with the pharmacological tools utilized in this study would also enable conclusions to be drawn as to whether the 5-HT_2 receptors are involved in processing afferent information from the bladder/urethra or efferent outflow to the bladder/urethra, or both. Of interest would be to investigate whether 5-ht_{5A} receptors are physiologically involved in mediating the effects observed on EUS-EMG activity as molecular evidence demonstrates labelling of 5-ht_{5A} receptors on motorneurones directly innervating the external urethral sphincter (Xu *et al.*, 2007).

From a clinical perspective, there is currently no evidence for the direct use of compounds acting at the 5-HT_2 receptor in the treatment of lower urinary tract dysfunctions. However, the present data may prove to be an important benchmark for the use of 5-HT_2 receptors as potential therapeutic targets. Activation of the 5-HT_{2A} receptor subtype lead to a pronounced increase in external urethral sphincter activity and this was

indicative of contraction of the striated sphincter muscles. As previously described, there is a dense accumulation of 5-HT_2 receptors in the Onuf's nucleus in the rat (Thor *et al.*, 1993) which is responsible for the actions of the external urethral sphincter. Further, it has been found that activation of these receptors with a 5-HT_2 receptor agonist results in an increase in the guarding reflex that prevents urine leakage. On the other hand, the inhibitory actions of the 5-HT_{2C} receptor subtype on the micturition reflex could be deemed as a model for the improvement in bladder capacity (demonstrated by the increase in volume threshold). Ideally, it would have been useful to apply the 5-$HT_{2C/2A}$ receptor agonists to a model of bladder irritation thus mimicking some of the overactive bladder symptoms. Therefore this current preclinical study, demonstrating the excitatory actions of 5-HT_{2A} receptors on the external urethral sphincter and the inhibitory actions of the 5-HT_{2C} receptors on the micturition reflex provides similar evidence to those observed following the use of the dual selective serotonin/noradrenaline reuptake inhibitor (SNRI) duloxetine which has been widely investigated for its use in the treatment of women with stress urinary incontinence (see Jost & Marsalek, 2004). Similar to the data from the current study, duloxetine has been observed to cause a pronounced increase in urethral sphincter activity during the storage phase of the micturition cycle (Thor & Katofiasc, 1995). Additionally, duloxetine was also observed to increase bladder capacity and striated sphincter activity in an anaesthetized acetic acid cat model thus emphasizing the value of its use in the treatment of overactive bladder symptoms.

In conclusion, the present study provides a promising strategy for the possibility of 5-HT_2 receptors as potential targets for the treatment of disorders of the lower urinary tract including stress urinary incontinence and overactive bladder syndrome. Although it is not possible to directly translate the pharmacological data from the present rat study to humans due to the differences in bladder and urethral structures, there is evidence provided for the potential to manipulate this 5-HT receptor family which has been shown to facilitate or suppress micturition, in the current study. There is therefore a great capacity for growth in this field and the current study boosts this concept by further elucidating the role of 5-HT receptors in the control of micturition with great insight into their potential as therapeutic targets for lower urinary tract dysfunctions.

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Published work

5-HT_{2C} receptor agonist Ro 60-175 and its role in the control of micturition in anaesthetized female rats
(Presented at the Society for Neuroscience meeting; October 2006)

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5-HT_{2A} and 5-HT_{2C} receptors have been implicated in activation of the external urethral sphincter (EUS) and inhibition of the micturition reflex (MR) respectively in rats (Mbaki et al 2005, PA_2 online Vol3Issue4abst065P), while in guinea-pigs 5-HT_{2C} receptors activate the EUS and inhibit the MR (McMurray and Miner 2005, The FASEB Journal 19, A536). This study further investigates the role of these receptors in micturition in the rat.

EUS EMG activity, bladder and urethral pressure (UP) were recorded in urethane anaesthetised female Sprague-Dawley rats (250-300g). Drug effects were evaluated on volume (VT) and pressure (PT) threshold during MRs evoked by saline infusion (0.1 ml min^{-1}) into the bladder and on EUS EMG activity and UP at 80% of the bladder volume required to evoke a control MR. Ro 60-0175 (5-HT_{2C} agonist) was given i.v. as a bolus (300 μg kg^{-1}; Cmax ~ 50nM free plasma concentration) or 30 min infusion (27, 90 and 270 μg kg^{-1} min^{-1} targeting 10, 30, and 100 nM free plasma concentration). MDL 100907, ketanserin (5-HT_{2A} antagonists) and SB 242084 (5-HT_{2C} antagonist) were given i.v. (30 μg kg^{-1} bolus) prior to i.v. bolus Ro 60-0175. Blood samples were taken for analysis of plasma concentration of Ro 60-0175. All values are means ± S.E.M (n=5/6).

Ro 60-0175 (i.v. bolus) significantly increased EUS EMG activity, UP, VT, and PT in comparison to vehicle controls (22 ± 3 v 1 ± 1 V; 2 ± 0.2 v 0.1 ± 0.1 mmHg; 0.4 ± 0.1 v 0.2 ± 0.02 ml; 5.7 ± 1.6 v 0.5 ± 0.9 mmHg respectively). MDL 100907 and ketanserin blocked EUS EMG activity but not UP, VT or PT effects of Ro 60-0175. SB 242084 blocked the EUS EMG and PT but not the UP effects of Ro 60-0175 and reversed the increase in VT to a decrease. Ro 60-0175 (27 and 90 μg kg^{-1} min^{-1}) had no effect on EUS EMG activity or UP compared with saline control rats, whilst 270 μg kg^{-1} min^{-1} significantly increased EUS EMG activity (11 ± 1 v 0.2 ± 0.1 V). Ro 60-0175 (27 μg kg^{-1} min^{-1}) significantly increased VT and PT whilst 90 and 270 μg kg^{-1} min^{-1} significantly decreased VT.

These data suggest that whilst 5-HT_{2C} receptor activation inhibits the MR, both 5-HT_{2A} and 5-HT_{2C} receptors are excitatory to the EUS. Neither receptor appears to be involved in the UP increase in rats. However, in guinea-pigs and dogs 5-HT_{2C} receptor activation does increase UP, which is more predictive for humans (Conlon et al. 2005 Soc. for Neurosci. Abstr 48.14). Ro 60-0175 appears to activate 5-HT_{2A} as well as 5-HT_{2C} receptors at higher concentrations.

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THE EFFECT OF 5-HT_{2C} RECEPTOR AGONIST Ro 60-0175 IN THE CONTROL OF THE URETHRA AND MICTURITION IN ANAESTHETISED FEMALE RATS
(Presented at the Physiological Society meeting; July 2006)

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In rats, stimulation of 5-HT_{2C} receptors caused inhibition of the micturition reflex, while stimulation of 5-HT_{2A} receptors caused activation of the external urethral sphincter (EUS); (Mbaki *et al.* 2005). However, in guinea-pigs using the selective 5-HT_{2C} receptor agonist Ro 60-0175, it was shown that stimulation of 5-HT_{2C} receptors caused activation of the EUS (McMurray & Miner. 2005). The present experiments were carried out to further investigate the effects of Ro 60-0175 on EUS-EMG activity, urethral pressure and the micturition reflex in the rat.

Experiments were performed on spontaneously breathing female Sprague-Dawley rats (250-300g) anaesthetized with isofluorane (5% in 100% oxygen) and maintained with urethane (1.2 g kg^{-1}, i.v.). Simultaneous recordings of EUS-EMG activity, urethral and bladder pressures, and carotid arterial blood pressure were made. Micturition reflexes were evoked by saline infusion (0.1 ml min^{-1}) into the bladder. All substances were given as i.v. bolus doses. Changes were compared with vehicle controls for baseline EUS-EMG activity and urethral pressure by two-way ANOVA and the micturition reflex by unpaired Student's *t*-test. All values are expressed as means ± S.E.M. $P < 0.05$ was considered to be significant.

Ro 60-0715 (300 μg kg^{-1}, n=5) caused a significant increase in baseline EUS-EMG activity and urethral pressure (22 ± 3 v 1 ± 1 V & 2 ± 0.2 v 0.1 ± 0.1 mmHg). The selective 5-HT_{2C} receptor antagonist SB 242084 (30 μg kg^{-1}, n=5) blocked the effects of Ro 60-0175 on EUS-EMG activity but failed to block the increase in urethral pressure. The 5-HT_{2A} receptor antagonists MDL 100907 and ketanserin (30 μg kg^{-1}, n=5) also blocked the effects of Ro 60-0175 on EUS-EMG activity but failed to block the increase in urethral pressure. Ro 60-0175 significantly increased bladder threshold pressure (5.7 ± 1.6 v 0.5 ± 0.9 mmHg), residual volume (0.2 ± 0.04 v 0.01 ± 0.01ml) and volume threshold (0.4 ± 0.1 v 0.2 ± 0.02 ml). SB 242084 significantly decreased the effects of Ro 60-0175 on bladder threshold pressure and volume threshold. MDL 100907 and ketanserin failed to block the effects of Ro 60-0175 on bladder threshold pressure, residual volume and volume threshold.

These data indicate that activation of either 5-HT_{2A} or 5-HT_{2C} receptors can cause excitation of the EUS in the rat. The data above also confirms an inhibitory role of 5 HT_{2C} receptors in the control of micturition. However, the receptor subtype by which 5 HT_2 receptor agonists cause an increase in urethral pressure in the rat (Mbaki *et al.* 2005) remains to be determined. Interestingly in guinea-pigs and dogs it has been shown that 5-HT_{2C} receptor activation increases UP, which is more predictive for humans (Conlon *et al.* 2005).

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THE ROLE OF 5-HT_2 RECEPTORS SUBTYPES IN THE CONTROL OF THE URETHRA AND MICTURITION IN ANAESTHETISED FEMALE RATS
(Presented at the British Pharmacological Society meeting; December 2005)

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5-HT receptors are implicated in the control of the bladder, however little is known of their role in control of the urethra. Nevertheless, 5-HT_2 receptor activation, by mCPP was reported to increase cavernous nerve activity (Steers & De Groat, 1989). The present experiments were carried out to investigate the effect of the 5-HT_2 receptor agonists, mCPP, Ro 60-0175, DOI (see Knight *et al.* 2004) and WAY 161503 (Cryan *et al.* 2000) on urethral pressure and sphincter (EUS) EMG activity and the micturition reflex.

Simultaneous recordings of EUS-EMG activity, urethral and bladder pressure, BP and HR were obtained from urethane-anaesthetised (1.2 g kg^{-1}) spontaneously breathing female Sprague-Dawley rats (200-300g). Micturition reflexes were evoked by saline infusion (0.1 ml min^{-1}) into the bladder. All test substances were given i.v. Changes in EUS-EMG activity, urethral and bladder pressure, BP and HR were compared with those in vehicle controls using two-way ANOVA and the least significant difference test. Changes in the micturition reflex were compared with vehicle controls using unpaired Student's *t*-test. All values are expressed as the mean ± s.e.mean. $P < 0.05$ was considered to be significant.

WAY 161503, Ro 60-0175, mCPP, (300 μg kg^{-1}, n=5) and DOI (100 μg kg^{-1}, n=5) each caused a significant increase in baseline EUS-EMG activity (179± 9%, 150±13%, 166±17%, & 198±15%). Only WAY 161503, Ro 60-0175 and mCPP significantly increased urethral pressure (10±2%, 13±2% & 24±2%). The selective 5-HT_{2A} receptor antagonist (Knight *et al.* 2004) ketanserin (30 μg kg^{-1}, n=5), blocked the effects of either WAY 161503 or DOI on EUS-EMG but not the ability of WAY 161503 to increase urethral pressure. The selective 5-HT_{2A} receptor antagonist, MDL 100907 (30 μg kg^{-1}, n=3), also antagonised this effect of DOI. mCPP blocked the micturition reflex, whereas WAY 161503 and Ro 60-0175 significantly increased bladder threshold pressure (25±11% & 90±33%) and volume threshold (55±13% & 71±15%) and DOI decreased only volume threshold (-35±7%). Ketanserin potentiated the effects of WAY 161503 on the volume but not pressure threshold and blocked the effect of DOI on volume threshold. MDL 100907 also blocked the effect of DOI on volume threshold.

These data indicates that activation of 5-HT_{2A} receptors evokes excitation of the external urethral sphincter, whereas it would seem that 5-HT_{2C} receptor activation causes constriction of urethral smooth muscle and inhibits the micturition reflex.

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Role of 5-HT_{2C} receptors in the control of micturition in anaesthetized female rats (Presented at the International Union of Physiological Sciences meeting; April 2005)

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Rats (250g) were anaesthetised with urethane (1.2g kg^{-1} i.v.); bladder and urethral pressures, sphincter (EUS)-EMG, BP and HR were recorded. The 5-HT_{2C} agonists WAY 161503 or mCPP (300 μg kg^{-1}; n = 5, i.v.) caused a significant increase in baseline EUS-EMG activity of 202 ± 15 % & 124 ± 22%. WAY 161503 had no effect on urethral pressure, whereas mCPP significantly increased the urethral pressure by 26 ± 6 %. WAY 161503 significantly increased the residual volume (62 ± 23 %), while mCPP completely blocked the distension evoked micturition reflex. Both WAY 161503 and mCPP (300 μg kg^{-1}, n = 3, 5 μl) given intracerebroventricularly (i.c.v.) and intrathecally (i.t.) had no effect on baseline variables, however on the reflex both drugs caused an increase in volume threshold and residual volume. In the presence of the peripheral 5-HT_2 antagonist BW501C67 (100 μg kg^{-1}, n = 6, i.v.) the effects of mCPP (i.v.) on EUS-EMG and urethral pressure tended to be potentiated (+569 ± 311% & +11 ± 11%). These data suggest that 5-HT_{2C} receptors in the brain and sacral spinal cord play an inhibitory role in the control of micturition while the ability of these agonists to increase baseline EUS-EMG is due to a peripheral site of action, probably not related to activation of 5-HT_2 receptors. Supported by a BBSRC collaborative studentship

Appendix (Chapter 7)

Determination of the relative expression levels of 5-HT_2 receptors in the rat brain, bladder and urethra

* The work in this chapter was carried out courtesy of Caroline Tolley (Discovery Biology, Pfizer, UK) who was investigating the sensitivity of the Roche TaqMan qRT-PCR kit.

7.1 Introduction

Pharmacological characterization of 5-HT_2 receptor subtypes revealed a role of 5-HT_2 receptors in the lower urinary tract (see chapters 3 & 4). It was concluded that 5-HT_{2A} receptors were excitatory on the external urethral sphincter and the micturition reflex and 5-HT_{2C} receptors were inhibitory on the micturition reflex. Additionally, it was concluded that activation of 5-HT_{2B} receptors caused contraction of the urethral smooth muscle.

5-HT_2 receptors have been well characterised at the molecular level with both central and peripheral distribution of the receptor. Studies have revealed 5-HT_{2A} receptor distribution in the CNS specifically in the cortical areas of the brain (Pazos *et al.*, 1985, 1987; López-Giménez *et al.*, 1997). Additionally, polymerase chain reaction (PCR) and southern hybridization analysis studies revealed 5-HT_{2A} receptor mRNA in the spinal cord of the rat, cat, monkey and human tissue (Helton *et al.*, 1994). 5-HT_{2B} receptors were found to be mainly located in peripheral tissues including the adult gut and cardiovascular system (Loric *et al.*, 1992). Some studies also provided evidence for a central distribution of 5-HT_{2B} receptors including cerebellum, cortex, hippocampus, amygdala and the spinal cord (Choi & Maroteaux, 1996; Helton *et al.*, 1994). With regards to the 5-HT_{2C} receptor subtype, distribution of the receptor has mainly been

observed in the central nervous system (both in the brain and spinal cord), with low to absent expression of the receptor in peripheral tissues (Helton *et al.*, 1994).

To my knowledge, there is no evidence in the literature examining the gene expression of the different 5-HT_2 receptor subtypes in the lower urinary tract. RT-PCR was therefore employed to identify the gene expression of all three 5-HT_2 receptor subtypes in the bladder, urethra and brain of female Sprague Dawley rats. This was done using the Roche TaqMan qRT-PCR kit. Performing this expression study therefore provided molecular evidence and support for the pharmacological observations made in the previous chapters regarding the involvement of 5-HT_2 receptors in the control of the lower urinary tract.

Below is a brief description of the PCR technique utilized in this study.

7.1.1 Polymerase Chain Reaction

PCR may be defined as a biochemical and molecular biology technique that enables the replication of DNA without the use of a living organism. PCR uses a naturally occurring nuclease enzyme, polymerase, to catalyze the regeneration of DNA, with a chain reaction that eventually exponentially amplifies the target DNA (Elrich, 1989). PCR is mainly used in gene technology for a broad variety of experiments and analyses, such as hereditary disease detection, cloning of genomic sequences, detecting viral pathogens as well as genetic fingerprinting (see Garcia & Ma, 2005).

The three steps that are repeated over and over again to exponentially amplify the target DNA include denaturation, annealing and extension. The process of denaturation requires heating of the template DNA mixture to a specific temperature (e.g. 90-95^{0}C), thus allowing the DNA double strand to denature and separate into two separate strands. This is followed by the annealing process which involves cooling the template DNA mixture (50-60^{0}C) to allow the primers (a pair of synthesized short DNA segments consisting of sense and antisense) to anneal to the right portion of the separated template and enable the DNA polymerase to begin copying the template, thus building new complimentary DNA (cDNA). The temperature is thereafter raised (~72^{0}C) and the third and final step, extension, takes place where the DNA polymerase links the loose deoxynucleotides to the primer and to each of the separated DNA strands in the appropriate sequence. The whole process of PCR requires repetition of the above three steps 30-40 times.

The most commonly used DNA polymerase for the PCR system is the Taq DNA polymerase. The enzyme is a naturally occuring enzyme that is derived from *Thermus aquaticus* and is known to be both thermostable and heat resistant. Taq DNA polymerase has a 5′- 3′ nuclease activity but lacks a 3′- 5′ proofreading exonuclease activity. These properties of Taq DNA polymerase enable the amplification reaction to run through heat resistant cycles with no interruptions. Additionally, the use of the enzyme significantly improves specificity and sensitivity of the reaction, as well as increasing the yield and length of the products (see Garcia & Ma, 2005).

7.1.2 Reverse Transcriptase PCR (RT-PCR)

RT-PCR is the method of choice for detection of mRNA (see Bustin, 2000), as it is the most sensitive and reliable technique when compared to Northern blot analysis and RNase protection assay. In RT-PCR, RNA and not DNA is used as the template, and the reverse transcriptase enzyme is needed to transcribe RNA to cDNA before the initiation of PCR (see Garcia & Ma, 2005). RT-PCR can be used to quantify mRNA levels from very small samples, and due to its sensitivity may be used to quantify RNA from a single cell. Quantitating RT-PCR enables a comparison of the amount of a given mRNA in two different samples to be made (see Luftalla & Uze, 2006). For each sample, the amount of the specific mRNA is given relative to the amount of reference mRNA that is constant in the two samples.

Real-time PCR enables data collection throughout the PCR process rather than at the end of the process. Reactions in real-time PCR are therefore characterised by the point in time during cycling where amplification of a target is first detected (threshold), rather than the amount of target accumulated after a fixed number of cycles. Introduction of fluorogenic probes that use the 5′ nuclease activity of Taq DNA polymerase has greatly improved real-time systems for PCR. These fluorogenic probes enable the detection of only specific amplification products by emmiting a fluorescence signal. In the initial cycles of PCR, there is little change in the fluorescence signal and this is thus defined as the baseline. When an accumulated target is detected, an increase in flourescence is observed in the cycle.

mRNA is quantitated either as relative or absolute. Relative quantitation analyses compare changes in gene expression in a given sample relative to another reference sample. In addition to the reference sample, a calibrator is required to create a series of dilutions. These series of dilutions can be used to contstruct a standard curve where a correlation plot is generated by subjecting the dilutions to amplification and real-time fluorescence detection, then plotting the known starting quantities against the measured threshold cycle (Ct) values.

It is important to note that the calibrator can be any nucleic acid as long as both the concentration and the length of the amplicon is known (see Bustin, 2000).
Absolute quantitation on the other hand allows the precise determination of copy number per cell, total RNA concentration, or unit mass of tissue. An absolute standard curve for each individual amplicon is required in order to ensure accurate reverse transcription and PCR amplification profiles.

7.1.3 How the detection system works in RT-PCR

The flourogenic probe enables the detection of a specific PCR product as it accumulates during PCR. The procedure of how this detection sytsem works is described below (see Figure 7.1 for diagram).

- An oligonucleotide probe is constructed containing a reporter (R) fluorescent dye on the 5′ end a quencher (Q) dye on the 3′ end. When intact, the proximity of the quencher dye greatly reduces the fluorescence emmitted by the reporter dye.

- If the target sequence is present, the probe anneals downstream from one of the primer sites and is cleaved by the 5′ nuclease activity of Taq DNA polymerase as the primer is extended.
- Cleavage of the probe involves, separating the reporter dye from the quencher dye, thus increasing the reporter dye signal, and removing the probe from the target strand thus allowing primer extension to continue to the end of the template strand. The presence of the probe therefore does not inhibit the overall PCR processs.
- Within each cycle, additional reporter dyes are cleaved from their respective probes, thus resulting in an increase in fluorescence intensity that is proportional to the amount of amplicon produced.

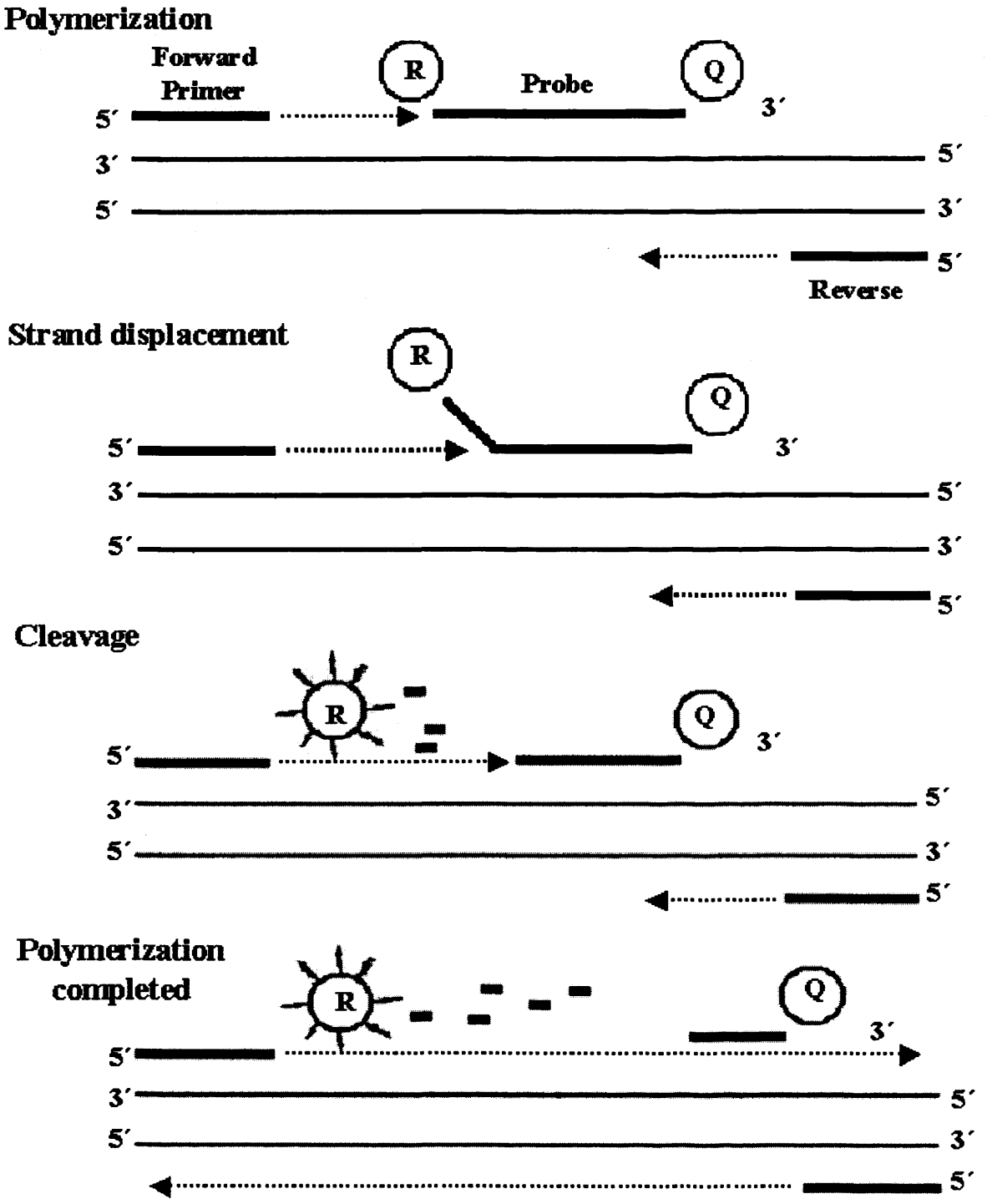


Figure 7.1 Schematic representation showing how the detection system works in RT-PCR as described above. Q represents the quencher dye on the 3′ end and R represents the flourescent reporter dye on the 5′ end.
(Adapted from http://docs.appliedbiosystems.com/pebiodocs/04371089.pdf)

7.2 Methods

Bladder and urethral tissues (both smooth and striated regions of the urethra) were obtained from female Sprague-Dawley rats (n = 3). The isolated tissues were stored in RNAlater (the recommended solution for "in-tissue" RNA preservation) at a temperature of 4^0C, until time for mRNA expression analysis. A Roche TaqMan qRT-PCR kit was used for mRNA detection. The kit consisted of sets of 90 pre-validated probes in a library format that was stored in the freezer ready for use. Roche's free web-based assay design centre was used to design primers specific for the $5HT_2$ receptors (5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}) in order to attain assay specificity. The online design centre also suggested which probe to use for each of the $5HT_2$ receptors investigated.

The isolated rat tissues of the bladder and urethra, as well as rat brain were used to extract RNA, manufacture cDNA and consequently determine expression levels of the $5HT_2$ receptors in each tissue isolated. From carrying out serial dilutions, varying concentrations of rat brain were used to construct a standard curve. The standard curve was used to calculate relative expression of the 5-HT_2 receptor subtypes in the brain, bladder and urethra.

7.3 Results

7.3.1 5-HT_{2A} receptor expression

Graph showing the relative expression levels of 5-HT_{2A} receptor mRNA in the rat urethra, bladder and brain tissue is shown in Figure 7.2a. 5-HT_{2A} receptor mRNA was observed to be low to absent in the rat urethra. However, 5-HT_{2A} receptor mRNA was observed to be abundantly expressed in the rat bladder relative to the rat brain and rat urethra (Table 7.2b).

7.3.2 5-HT_{2B} receptor expression

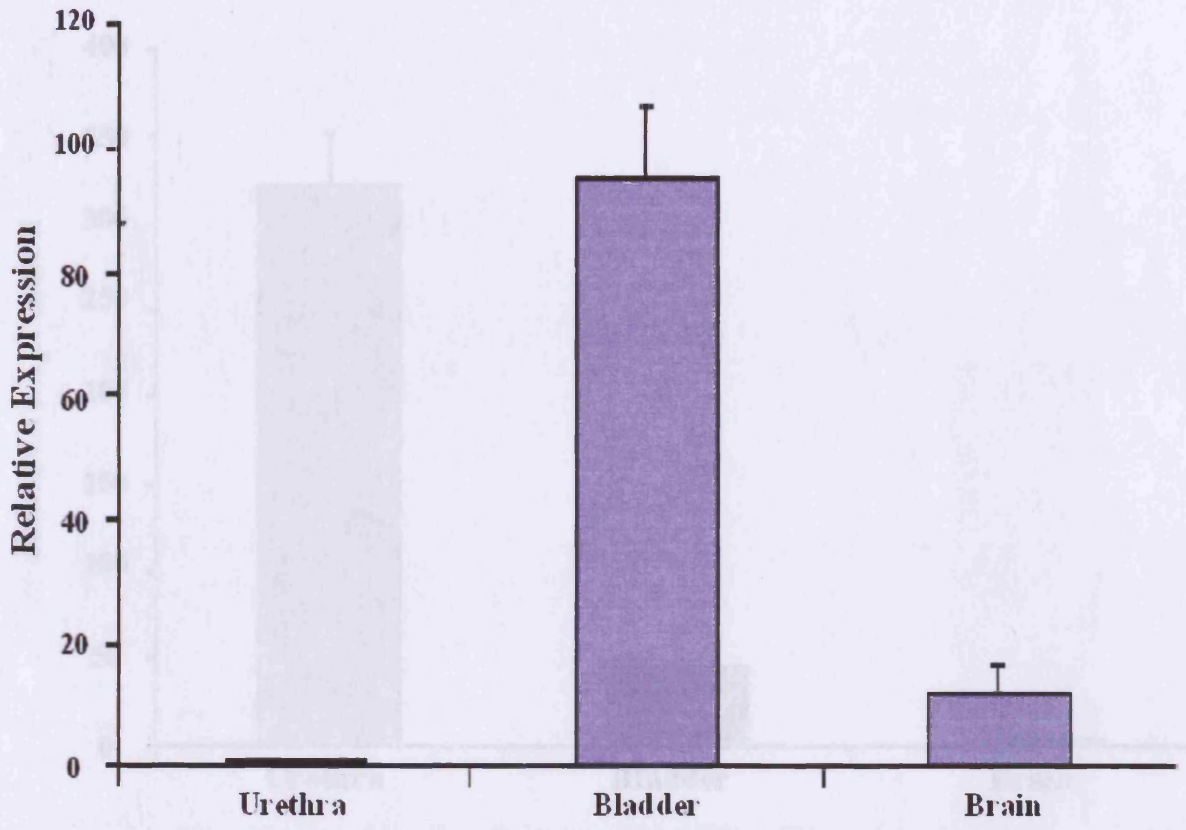
Graph showing the relative expression levels of 5-HT_{2B} receptor mRNA in the rat urethra, bladder and brain tissue is shown in Figure 7.3a. The relative expression of 5-HT_{2B} receptor mRNA was observed to be highest in the rat urethra relative to both the rat bladder and rat brain (Table 7.3b).

7.3.3 5-HT_{2C} receptor expression

Graph showing the relative expression levels of 5-HT_{2C} receptor mRNA in the rat urethra, bladder and brain tissue is shown in Figure 6.4a. 5-HT_{2C} receptor mRNA was observed to be absent in the rat urethra and bladder but was observed to be abundantly expressed in the rat brain (Table 7.4b).

$5HT_{2A}$ receptor expression

(a)



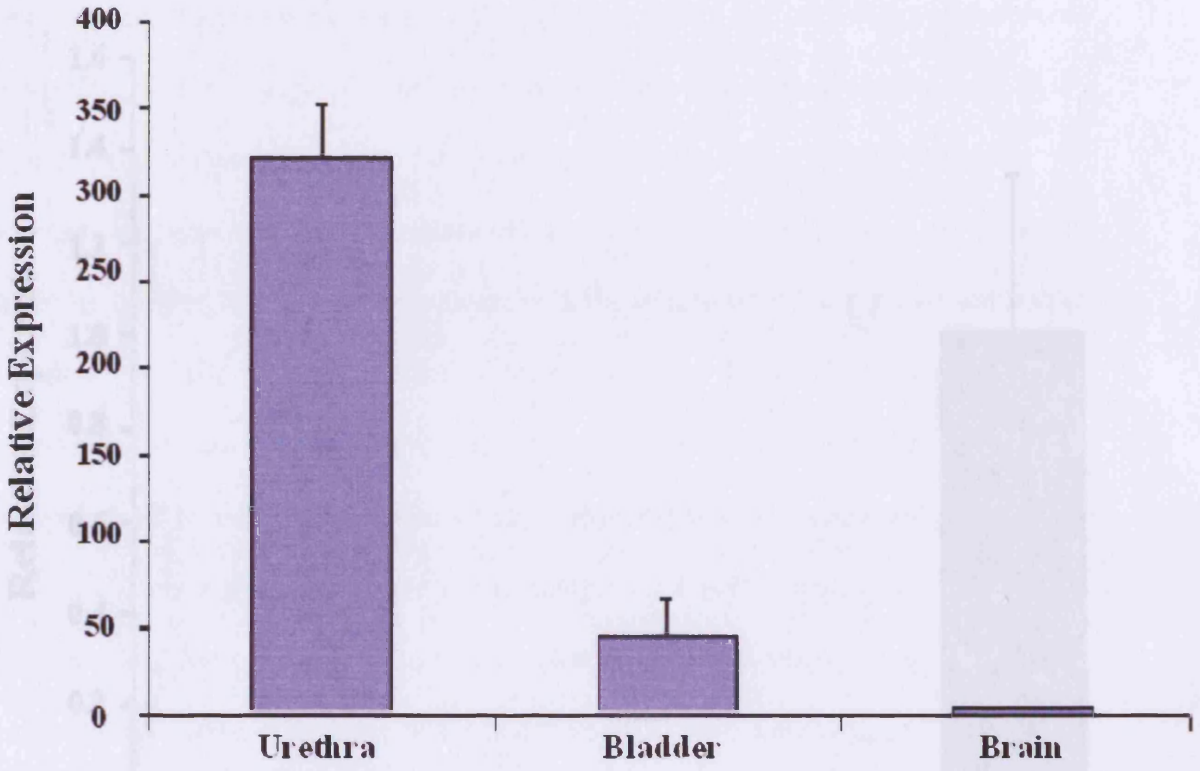
(b)

Tissue	$5HT_{2A}$ expression relative to brain	Standard deviation
Urethra	1	0.4
Bladder	96	11
Brain	12	5

Figure 7.2 **(a)** Histograms showing relative $5HT_{2A}$ receptor expression in the rat urethra, bladder and brain. **(b)** Table summarising mRNA concentration of 5-HT_{2A} receptor expression in the rat urethra, bladder and brain.

$5HT_{2B}$ receptor expression

(a)



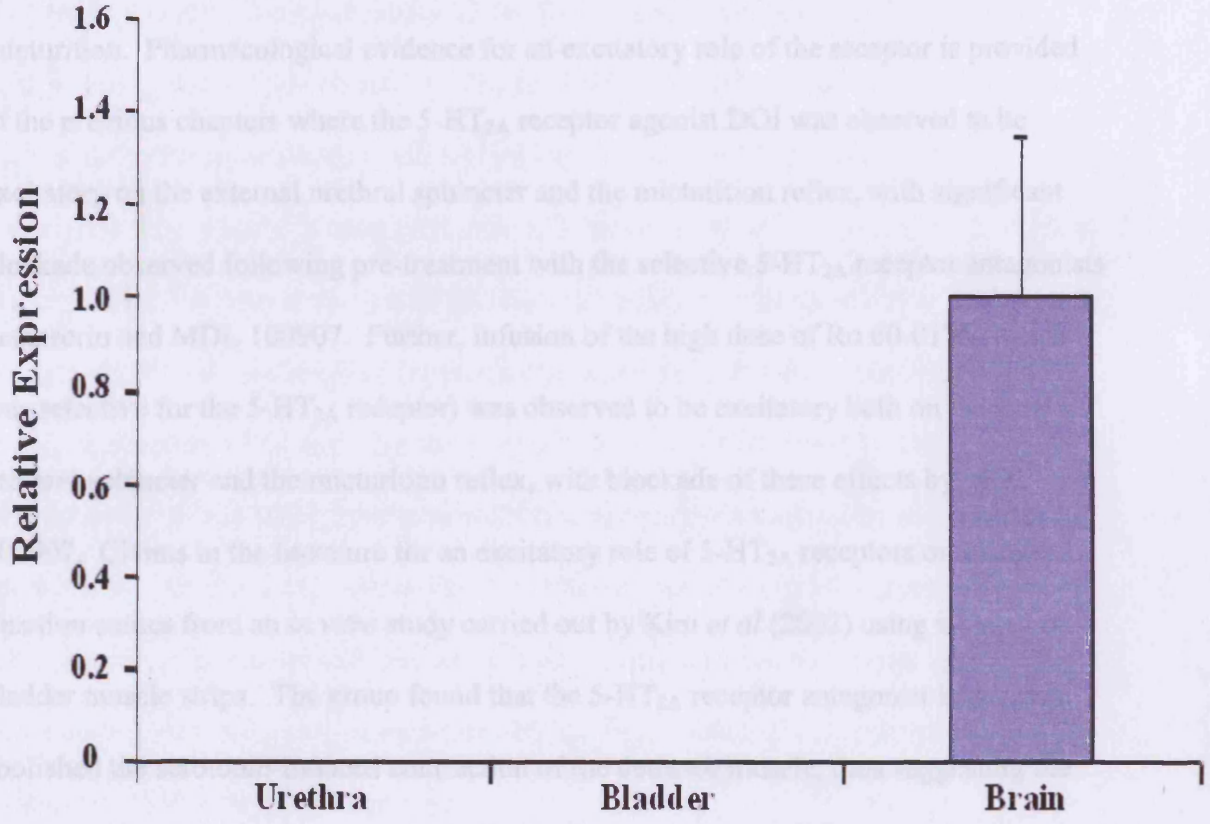
(b)

Tissue	$5HT_{2B}$ expression relative to brain	Standard deviation
Urethra	321	31
Bladder	46	20
Brain	1	0.2

Figure 7.3 **(a)** Histograms showing relative $5HT_{2B}$ receptor expression in the rat urethra, bladder and brain. **(b)** Table summarising mRNA concentration of 5-HT_{2B} receptor expression in the rat urethra, bladder and brain.

5HT$_{2C}$ receptor expression

(a)



(b)

Tissue	5HT$_{2C}$ expression relative to brain	Standard deviation
Urethra	0	0
Bladder	0	0
Brain	1	0.3

Figure 7.4 **(a)** Histograms showing relative 5HT$_{2C}$ receptor expression in the rat urethra, bladder and brain. **(b)** Table summarising mRNA concentration of 5-HT$_{2C}$ receptor expression in the rat urethra, bladder and brain.

7.4 Discussion

In the present data, 5-HT_{2A} receptor mRNA was observed to be low to absent in the rat urethra, but highly expressed in the rat bladder relative to the rat brain. These observations therefore help in further defining an excitatory role for 5-HT_{2A} receptors in micturition. Pharmacological evidence for an excitatory role of the receptor is provided in the previous chapters where the 5-HT_{2A} receptor agonist DOI was observed to be excitatory on the external urethral sphincter and the micturition reflex, with significant blockade observed following pre-treatment with the selective 5-HT_{2A} receptor antagonists ketanserin and MDL 100907. Further, infusion of the high dose of Ro 60-0175 (which was selective for the 5-HT_{2A} receptor) was observed to be excitatory both on the external urethral sphincter and the micturition reflex, with blockade of these effects by MDL 100907. Claims in the literature for an excitatory role of 5-HT_{2A} receptors on bladder function comes from an *in vitro* study carried out by Kim *et al* (2002) using isolated rat bladder muscle strips. The group found that the 5-HT_{2A} receptor antagonist ketanserin abolished the serotonin-induced contraction of the detrusor muscle, thus suggesting the involvement of 5-HT_{2A} receptors in mediating these excitatory effects. A lack of evidence of 5-HT_{2A} receptor mRNA expression in the rat urethra concurs with the *in vivo* results obtained in chapter 3. The data showed no significant increases on urethral pressure following administration of DOI.

5-HT_{2B} receptor mRNA expression was mainly observed in the rat urethra relative to the bladder and brain. This should however be approached with caution as there was no distinction made regarding receptor expression between the two urethral muscles i.e.

smooth or striated muscle. However, there is evidence in the literature demonstrating peripheral distribution of the 5-HT_{2B} receptor in stomach, intestine and pulmonary smooth muscle (Foguet *et al.*, 1992; Kursar *et al.*, 1994; Baxter *et al.*, 1994). Therefore, if 5-HT_{2B} receptor mRNA expression primarily occurred in the urethral smooth muscle, this would ascertain the involvement of 5-HT_{2B} receptors in urethral smooth muscle contraction as observed in chapter 3. The results from this study also concur with other studies in the literature where low to absent expression of 5-HT_{2B} receptor mRNA occurred in the rat brain (Kursar *et al.*, 1994; Schmuck *et al.*, 1994) which would explain the lack of evoked responses by 5-HT_{2B} receptor agonist and antagonist BW 723C86 and RS 127445 respectively on the micturition reflex as it is known to be mediated via a central mechanism. It should also be noted that there was a technical limitation regarding the expression of the 5-HT_{2B} receptor mRNA as the rat brain tissue used to construct the standard curve in this study, showed very low expression of the 5-HT_{2B} receptor mRNA, hence deeming the results less reliable. It would therefore have been more appropriate to construct the standard curve using a more highly expressing 5-HT_{2B} receptor tissue such as stomach fundus smooth muscle.

5-HT_{2C} receptor mRNA expression was expressed abundantly in the rat brain relative to the bladder and urethra. These results concur with other studies in the literature where 5-HT_{2C} receptors have been identified in many parts of the rat, human and monkey brain (Abramowski *et al.*, 1995; López-Giménez *et al.*, 2001) with low to absent expression in the peripheral tissues. Several studies in the literature have deduced that the mechanism for micturition is mediated centrally (see Ramage, 2006) and therefore a correlation may

exist between the abundant expression of the 5-HT_{2C} receptor mRNA in the brain and its function in micturition.

The molecular evidence provided in this study, correlates well with the pharmacological results observed in the previous chapters with regards to the role of the three 5-HT_2 receptor subtypes in micturition. Although the results present insights into 5-HT_2 receptor mRNA expression in the lower urinary tract, linking mRNA receptor expression to receptor function should be approached with great caution. This is due to the fact that RNA processing events such as RNA editing may occur, thus altering the encoded receptor protein which may in turn alter the protein structure and physiological function of the receptor (Niswender *et al.*, 1998). This is especially the case with the 5-HT_{2C} receptor which undergoes RNA editing thus resulting in generation of multiple receptor isoforms with altered phospholipase C signal transduction (Niswender *et al.*, 1998).

7.5 Conclusion

In conclusion, from this study, molecular evidence was provided regarding expression of 5-HT_2 receptor subtypes in the lower urinary tract of the rat, with 5-HT_{2A} receptor mRNA expression observed in the bladder, 5-HT_{2B} receptor mRNA expression in the urethra and 5-HT_{2C} receptor mRNA expression observed in the brain. Findings from this study support the evidence provided in the previous chapters where insights into the role of the three 5-HT_2 receptor subtypes in the lower urinary tract were provided and confirmed following pharmacological characterisation of the receptors and their role in the lower urinary tract.